***Supplementary table IV.4 | Incorporation rates of "heavy" and "medium" isotopes of arginine and lysine in cells after 10 days of growth in SILAC media. "****Heavy" and "medium" isotopes were incorporated during cell growth. The rate describes the percentage of "heavy" and "medium" amino acid isotopes in the cells proteins. Cells were considered fully labeled since the incorporation rate was high (above 95 %).*

| | ***Fmr1$^{+}$ MEF*** | ***Fmr1$^{-}$ MEF*** |
|---|---|---|
| ***Arginine 6*** | 0.97 | 0.97 |
| ***Lysine 4*** | 0.97 | 0.97 |
| ***Arginine 10*** | 0.97 | 0.96 |
| ***Lysine 8*** | 0.97 | 0.97 |

***Supplementary table IV.5 | SILAC heavy/light mixing error.*** *Summary of unnormalized proportions of "heavy", "medium" and "light" labeled evidences compared to each other. The numbers of detected and quantified evidences are listed additionally.*

| | ***Median (M/L)*** | ***Median (H/L)*** | ***Median (H/M)*** | ***Detected evidences*** | ***Quantified evidences*** |
|---|---|---|---|---|---|
| ***Proteome Fmr1$^{+}$ MEF biological replicate 1*** | 1.11 | 1.06 | 0.96 | 47,722 | 31,032 |
| ***Proteome Fmr1$^{+}$ MEF biological replicate 2*** | 1.08 | 1.10 | 1.03 | 88,444 | 66,308 |
| ***Proteome Fmr1$^{-}$ MEF biological replicate 1*** | 1.06 | 1.14 | 1.08 | 64,590 | 50,407 |
| ***Proteome Fmr1$^{-}$ MEF biological replicate 1*** | 1.11 | 0.99 | 0.89 | 80,190 | 62,828 |
| ***Phosphoproteome Fmr1$^{+}$ MEF biological replicate 1*** | 1.15 | 1.12 | 0.97 | 30,014 | 20,097 |
| ***Phosphoproteome Fmr1$^{+}$ MEF biological replicate 2*** | 1.05 | 1.05 | 1.00 | 11,238 | 9,247 |
| ***Phosphoproteome Fmr1$^{-}$ MEF biological replicate 1*** | 1.09 | 1.15 | 1.06 | 27,781 | 22,034 |
| ***Phosphoproteome Fmr1$^{-}$ MEF biological replicate 1*** | 1.09 | 0.98 | 0.90 | 10,897 | 8,961 |

***Supplementary table IV.7 | Potential substrates of GSK-3β in Fmr1⁺ MEFs (class 3).*** *Table contains protein name of the potential substrate, presence of GSK-3 motif, overlap with known substrate [228], position of phosphorylated amino acid and phosphorylated residue.*

| Protein.names | *GSK-3 motif* | *Reported substrates* | *Position* | *Amino.acid* |
|---|---|---|---|---|
| ADP-ribosylation factor GTPase-activating protein 1 | | | 360 | S |
| Alpha-1-syntrophin | + | | 195 | S |
| Armadillo repeat protein deleted in velo-cardio-facial syndrome homolog | | | 141 | S |
| Catenin alpha-2 | | | 655 | S |
| Cyclin-dependent kinase 17 | | | 92 | S |
| Cytospin-B | + | | 355 | S |
| Cytospin-B | + | | 133 | S |
| Cytospin-B | | | 914 | S |
| E3 ubiquitin-protein ligase RNF213 | | | 79 | S |
| Filamin A-interacting protein 1-like | | | 809 | S |
| Filamin-A | + | | 16 | S |
| Formin-1 | + | | 616 | S |
| Formin-binding protein 1-like | | | 429 | S |
| Glucocorticoid-induced transcript 1 protein | | | 107 | S |
| Heat shock protein beta-1 | + | | 13 | S |
| Interferon regulatory factor 2-binding protein-like | | | 637 | S |
| Interferon-induced helicase C domain-containing protein 1 | | | 289 | S |
| Interferon-induced helicase C domain-containing protein 1 | + | | 302 | S |
| Interferon-induced, double-stranded RNA-activated protein kinase | | | 32 | S |
| Leiomodin-1 | + | | 550 | S |
| Lipid phosphate phosphohydrolase 1 | | | 271 | T |
| MARCKS-related protein | + | | 85 | T |
| Matrix metalloproteinase-23 | | | 240 | Y |
| Microtubule-associated protein 1B | + | + | 2030 | S |
| Mitogen-activated protein kinase kinase kinase MLT | | | 649 | S |
| Muscle-related coiled-coil protein | + | | 334 | T |
| Nestin | + | | 575 | S |
| Nestin | | | 728 | S |
| Nestin | + | | 731 | S |
| Nestin | + | | 963 | S |
| Nestin | + | | 169 | S |
| Nexilin | | | 16 | S |
| Palladin | + | | 987 | S |
| Paxillin | | | 302 | S |
| PDZ and LIM domain protein 5 | + | | 332 | S |
| Pleckstrin homology domain-containing family A member 1 | | | 70 | S |
| Plekha5 protein | | | 417 | S |
| Protein phosphatase 1 regulatory subunit 12A | + | | 422 | S |
| Protein prune homolog 2 | + | | 1876 | S |
| Protein prune homolog 2 | + | | 594 | S |
| Ras GTPase-activating protein-binding protein 2 | + | | 227 | T |
| Sequestosome-1 | + | | 269 | T |
| Serine-rich coiled-coil domain-containing protein 1 | + | | 375 | S |
| Signal-induced proliferation-associated 1-like protein 1 | | | 288 | S |

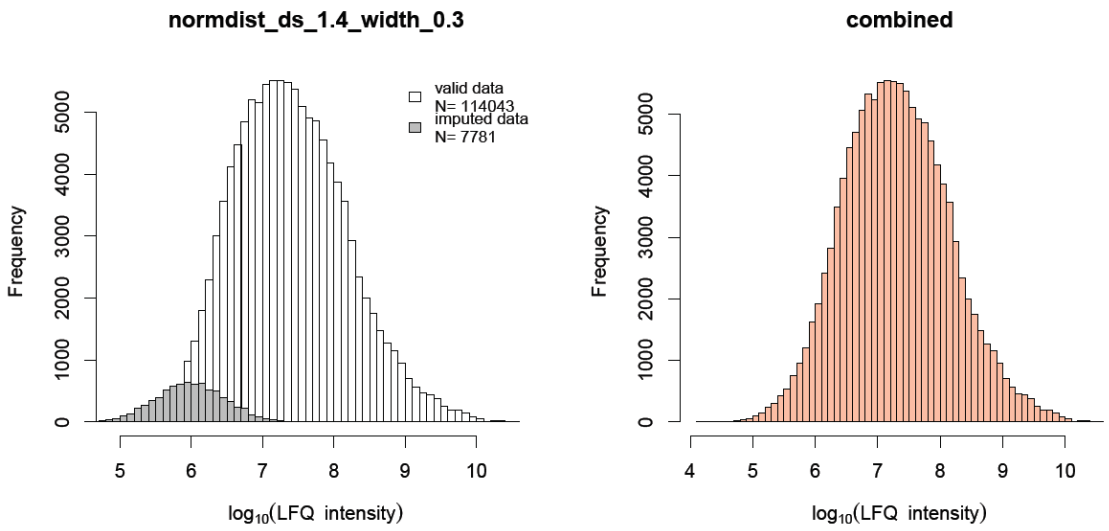
| Protein.names | GSK-3 motif | Reported substrates | Position | Amino.acid |
|---|---|---|---|---|
| Sodium bicarbonate cotransporter 3 | | | 247 | S |
| Sodium-coupled neutral amino acid transporter 1 | | | 52 | S |
| TBC1 domain family member 1 | | | 231 | S |
| Torsin-1A-interacting protein 1 | + | | 33 | S |
| Tropomyosin alpha-1 chain;Tropomyosin beta chain | | | 87 | S |
| Tumor protein D54 | | | 5 | S |
| Tumor protein D54 | + | | 12 | S |
| Tyrosine-protein kinase Fyn | | | 21 | S |
| Voltage-dependent P/Q-type calcium channel subunit alpha-1A | | | *2168* | S |

***Supplementary table IV.8 \| Potential substrates of GSK-3β in Fmr1$^{-}$ MEFs (class 3).*** *Table contains protein name of the potential substrate, presence of GSK-3 motif, overlap with known substrate [228], position of phosphorylated amino acid and phosphorylated residue.*

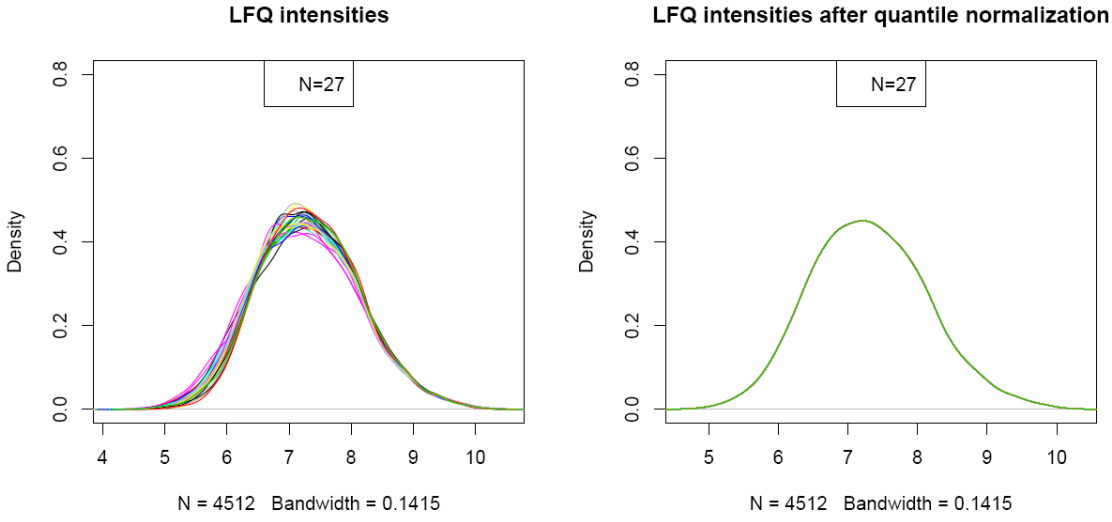
| **Protein.names** | ***GSK-3 motif*** | ***Reported substrates*** | ***Position*** | ***Amino.acid*** |
|---|---|---|---|---|
| 182 kDa tankyrase-1-binding protein | | | 1212 | S |
| 6-phosphofructokinase, liver type | | | 775 | S |
| Acetyl-coenzyme A synthetase, cytoplasmic | + | | 263 | S |
| Actin-binding protein anillin | + | | 180 | S |
| Actin-binding protein anillin | | | 444 | S |
| ADP-ribosylation factor GTPase-activating protein 2 | | | 431 | S |
| AF4/FMR2 family member 4 | + | | 1040 | S |
| Ahnak protein | | | 5596 | S |
| Ahnak protein | | | 576 | S |
| AP-2 complex subunit mu | | | 147 | S |
| AP-2 complex subunit mu | | | 154 | T |
| Apolipoprotein B receptor | + | | 420 | S |
| Apolipoprotein B receptor | + | | 484 | S |
| Apolipoprotein B receptor | | | 398 | S |
| Band 4.1-like protein 3 | + | | 495 | T |
| Calcium-regulated heat stable protein 1 | + | | 31 | S |
| Calcium-regulated heat stable protein 1 | + | | 42 | S |
| Catenin delta-1 | | + | 201 | T |
| Citron Rho-interacting kinase | + | | 1956 | S |
| Cyclin-dependent kinase 12 | | | 889 | T |
| Cyclin-dependent kinase 14 | | | 78 | S |
| Cytosine-specific methyltransferase | + | | 598 | S |
| Cytospin-A | | | 52 | T |
| Dedicator of cytokinesis protein 7 | | | 452 | S |
| Density-regulated protein | + | | 73 | S |
| Deoxynucleoside triphosphate triphosphohydrolase SAMHD1 | + | | 603 | T |
| Dihydropyrimidinase-related protein 2 | | + | 542 | S |
| Dihydropyrimidinase-related protein 3 | + | + | 101 | S |

| Protein.names | GSK-3 motif | Reported substrates | Position | Amino.acid |
|---|---|---|---|---|
| Disks large homolog 3 | | | 8 | S |
| Disks large-associated protein 5 | + | | 70 | S |
| DNA ligase | | | 210 | T |
| DNA ligase 1 | + | | 188 | S |
| DNA ligase 1 | + | | 51 | S |
| DNA replication licensing factor MCM2 | | | 140 | S |
| Drebrin-like protein | | | 273 | S |
| Drebrin-like protein | | | 295 | T |
| E1A-binding protein p400 | + | | 923 | S |
| Elongation factor 2 | | | 57 | T |
| Eukaryotic translation initiation factor 4 gamma 1 | + | | 1184 | S |
| FH2 domain-containing protein 1 | | | 645 | S |
| Filamin-A | | | 1731 | T |
| Filamin-A | + | | 1742 | T |
| Filamin-B | + | | 2478 | S |
| Fos-related antigen 2 | + | | 120 | S |
| Fructose-bisphosphate aldolase A | | | 36 | S |
| Gap junction alpha-1 protein | | | 306 | S |
| Heat shock protein HSP 90-alpha | | | 316 | S |
| Heat shock protein HSP 90-alpha | | | 318 | T |
| Heterogeneous nuclear ribonucleoproteins C1/C2 | | | 229 | S |
| Inner centromere protein | | | 284 | S |
| Interferon-activable protein 204 | | | 165 | S |
| Interferon-induced, double-stranded RNA-activated protein kinase | + | | 163 | S |
| Intersectin-1 | | | 166 | S |
| Junctional protein associated with coronary artery disease | | | 831 | S |
| Kinesin-like protein KIF15 | + | | 568 | S |
| Lamin-B1 | + | | 24 | S |
| Lamin-B1;Lamin-B2 | + | | 392 | S |
| Lamin-B1;Lamin-B2 | + | | 394 | S |
| Lmo7 protein | | | 927 | T |
| Metalloreductase STEAP3 | | | 20 | S |
| Microtubule-associated protein 1A | | | 667 | S |
| Microtubule-associated protein 1A | + | | 1606 | S |
| Microtubule-associated protein 1A | + | | 1580 | S |
| Microtubule-associated serine/threonine-protein kinase 4 | | | 1176 | S |
| MKL/myocardin-like protein 1 | + | | 6 | S |
| Multivesicular body subunit 12A | | | 168 | S |
| Myb-binding protein 1A | + | | 1164 | S |
| Myb-binding protein 1A | + | | 1253 | S |
| Myc box-dependent-interacting protein 1 | + | | 293 | S |
| Myosin phosphatase Rho-interacting protein | | | 1015 | S |
| NAD kinase | | | 62 | T |
| Nuclear factor interleukin-3-regulated protein | + | | 301 | S |
| Nuclear pore complex protein Nup107 | | | 83 | T |
| Nuclear pore complex protein Nup98-Nup96 | + | | 839 | S |
| Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1 | + | | 181 | S |
| Nucleolar RNA helicase 2 | | | 118 | S |

| Protein.names | GSK-3 motif | Reported substrates | Position | Amino.acid |
|---|---|---|---|---|
| Nucleolin | + | | 121 | T |
| Nucleophosmin | | | 252 | S |
| Nucleophosmin | + | | 217 | T |
| OTU domain-containing protein 7B | | | 100 | S |
| PDZ and LIM domain protein 2 | | | 210 | S |
| Phosphatidylinositol 4-kinase type 2-alpha | | | 462 | S |
| Prelamin-A/C | + | | 392 | S |
| Prelamin-A/C | | | 463 | S |
| Protein WWC2 | | | 999 | T |
| R3H domain-containing protein 2 | | | 871 | S |
| R3H domain-containing protein 2 | | | 283 | S |
| Rab11 family-interacting protein 5 | | | 174 | S |
| RAB23, member RAS oncogene family, isoform CRA_a | | | 197 | S |
| Radiation-inducible immediate-early gene IEX-1 | | | 31 | S |
| Ras and Rab interactor 1 | | | 446 | S |
| Receptor-interacting serine/threonine-protein kinase 2 | | | 176 | S |
| Receptor-interacting serine/threonine-protein kinase 2 | | | 178 | S |
| Receptor-interacting serine/threonine-protein kinase 2 | | | 364 | S |
| Replication factor C subunit 1 | + | | 155 | S |
| Reticulon-4 | + | | 16 | S |
| Rho guanine nucleotide exchange factor 2 | | | 93 | S |
| Rhotekin | | | 507 | S |
| SAM and SH3 domain-containing protein 1 | | | 400 | S |
| Septin-10 | | | 413 | S |
| Serine/arginine repetitive matrix protein 2 | + | | 964 | S |
| Serine/threonine-protein kinase SIK2 | | | 484 | T |
| Serine/threonine-protein kinase SIK3 | | | 221 | T |
| Sister chromatid cohesion protein PDS5 homolog B | + | | 1281 | S |
| Smith-Magenis syndrome chromosomal region candidate gene 8 protein homolog | | | 488 | S |
| Sorting nexin-7 | | | 8 | S |
| Spermatogenesis-associated protein 13 | | | 670 | S |
| Splicing factor 3B subunit 1 | + | | 223 | T |
| Srcap protein | + | | 45 | S |
| Supervillin | | | 407 | T |
| Target of rapamycin complex 2 subunit MAPKAP1 | | | 476 | S |
| Tensin-3 | + | | 1436 | S |
| Thymidine kinase, cytosolic | | | 15 | S |
| Transcription factor HIVEP2 | | | 764 | S |
| Tubulin alpha-1B chain | | | 48 | S |
| Tumor protein D54 | | | 5 | S |
| Ubiquitin-conjugating enzyme E2 O | + | | 836 | S |
| Uncharacterized protein C7orf50 homolog | + | | 180 | S |
| Versican core protein | | | 1622 | S |
| WD repeat and HMG-box DNA-binding protein 1 | | | 784 | S |
| Zinc finger CCCH domain-containing protein 11A | + | | 677 | S |
| Zinc finger protein GLI2 | | | 848 | S |

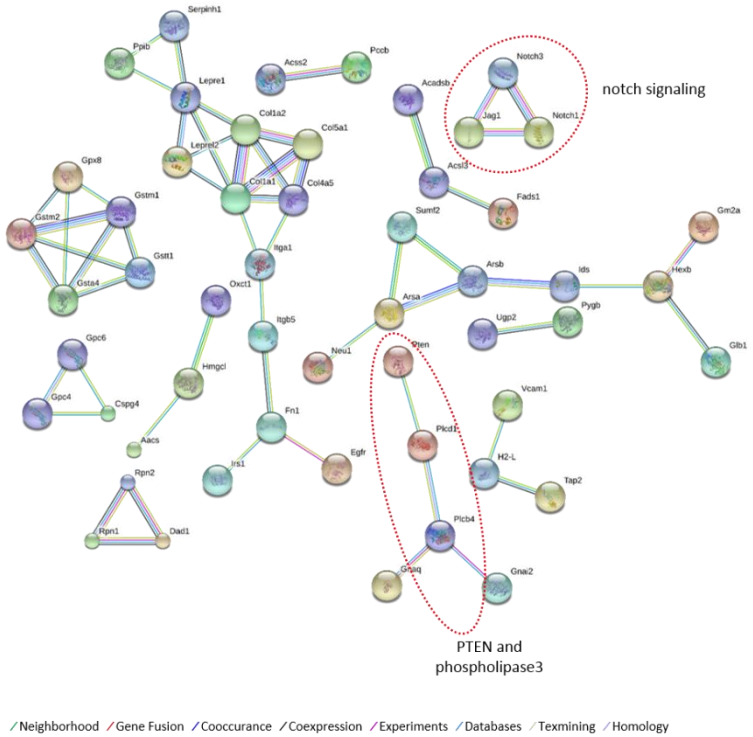



***Supplementary figure III.1 | Imputation of missing data.*** *Label-free intensities were log10-transformed and missing values were imputed to simulate protein abundances near the detection limit using values 1.4 for 'downshift' and 0.3 for width, respectively.*

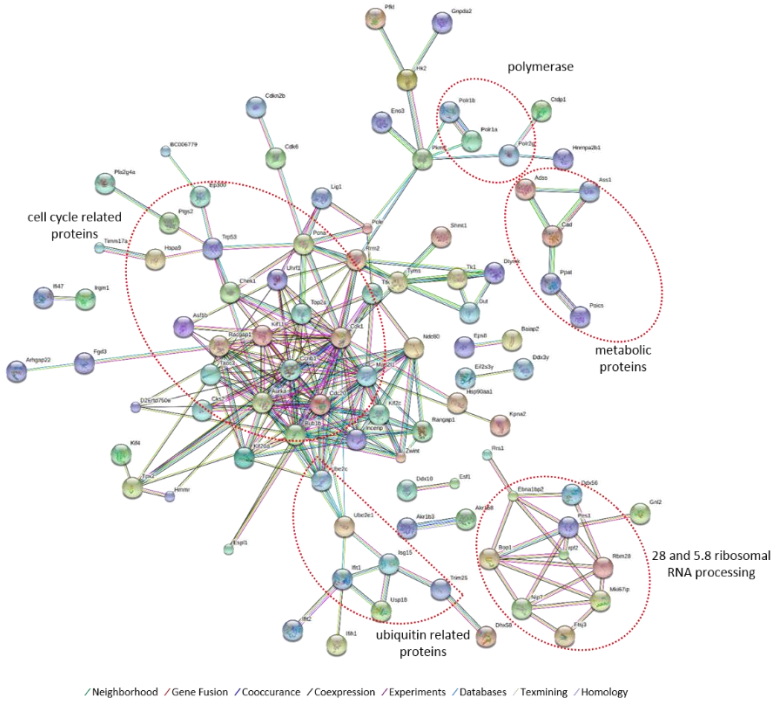



***Supplementary figure III.2 | Quantile normalization of LFQ intensities.*** *LFQ intensities across the different measurements were normalized according to the Quantile method using function 'normalize.quantiles' form the 'preprocessCore' R-package.*

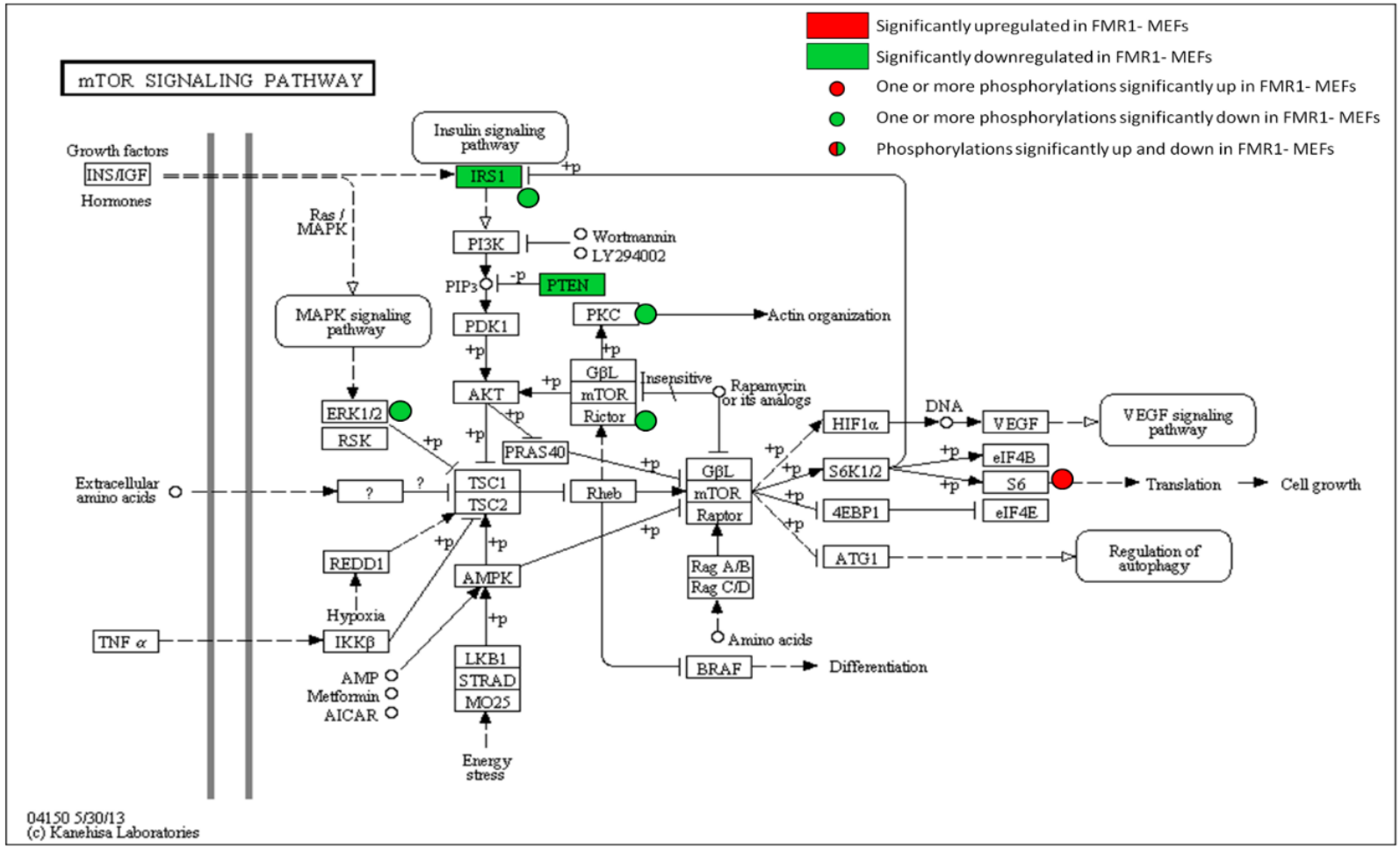
**A**



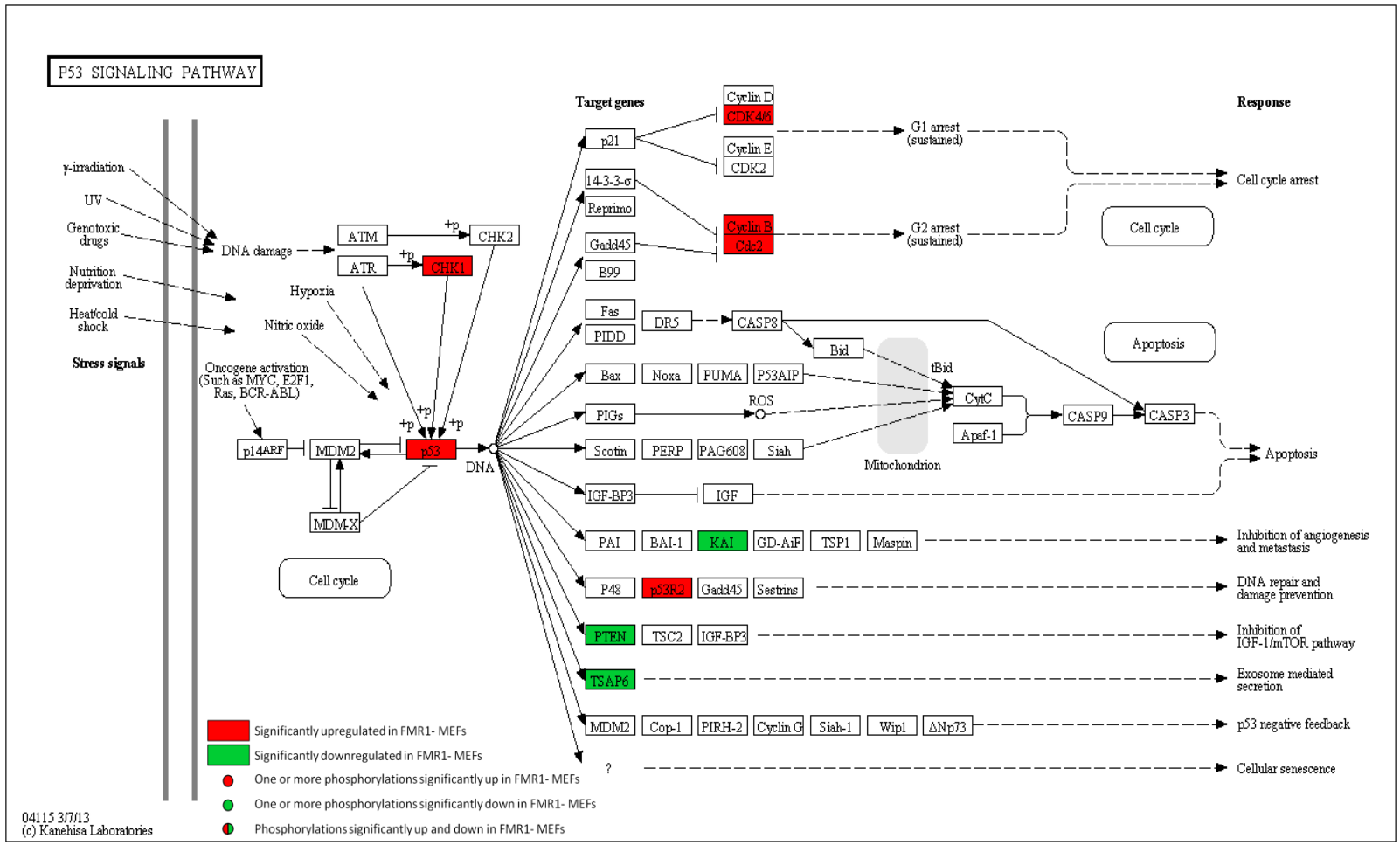

**B**



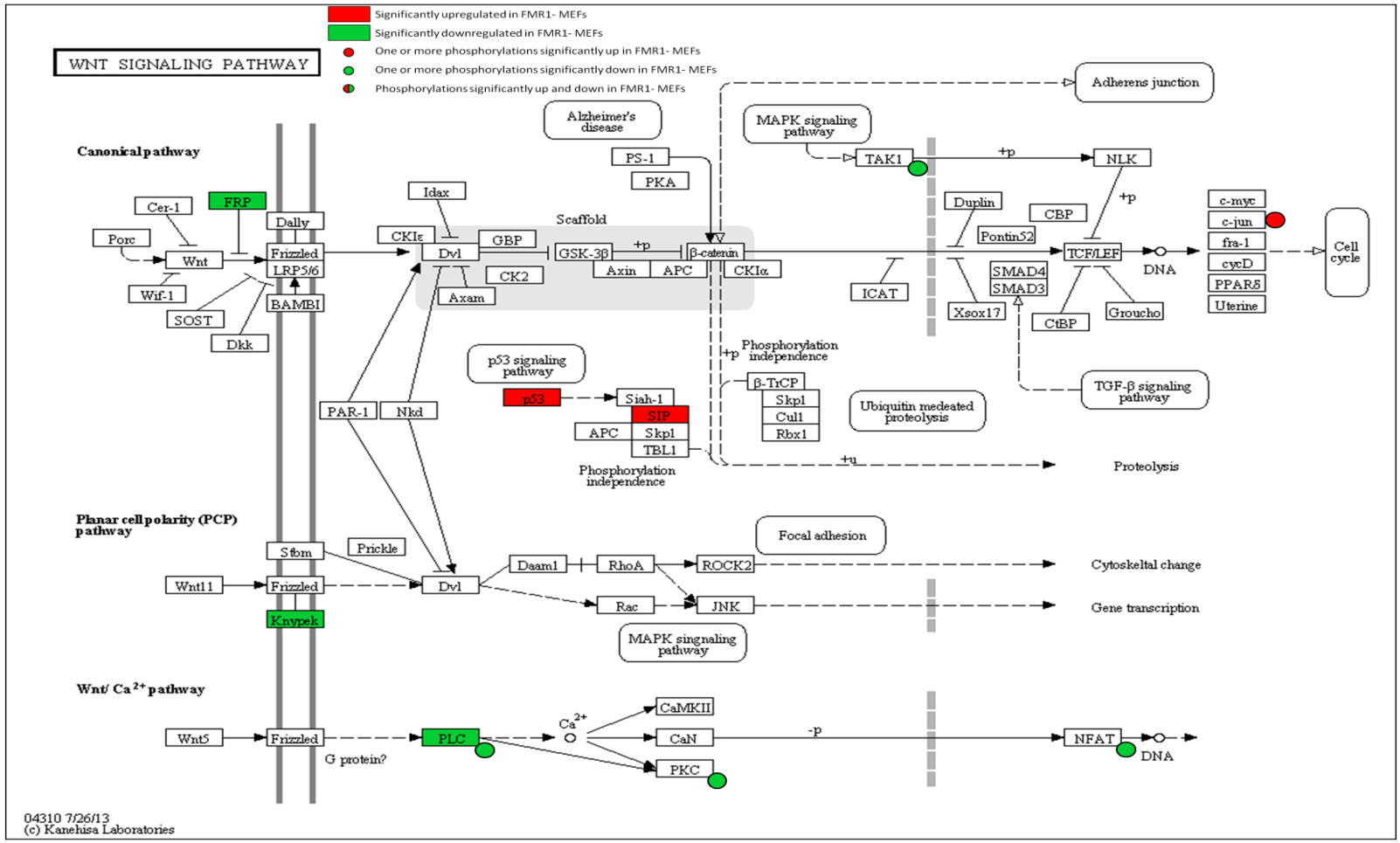

***Supplementary figure IV.1 | STRING analysis.*** *a) Network from Class 1-3 proteins with increased level; b) Network from Class 1-3 proteins with decreased level.*



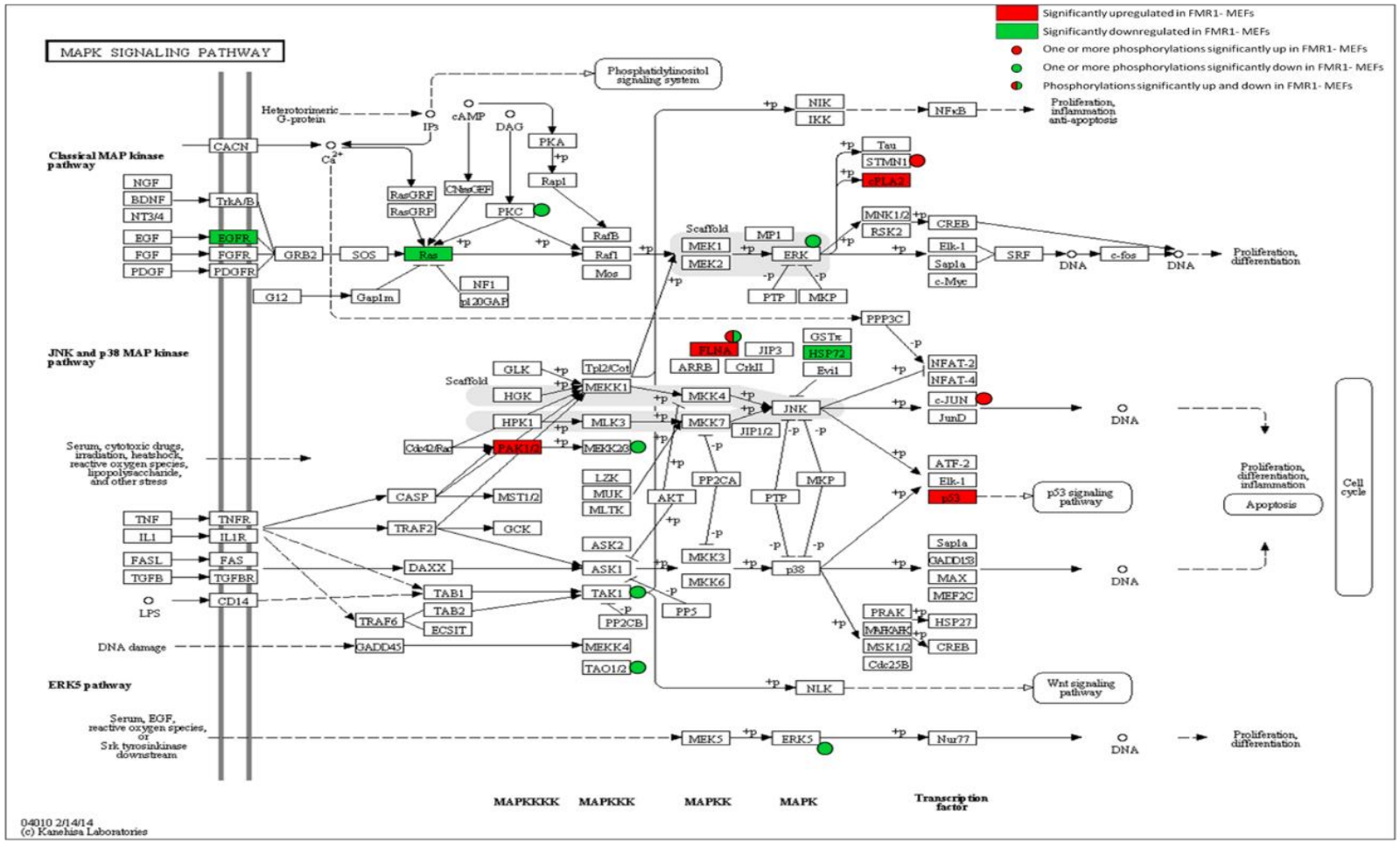

***Supplementary figure IV.2 | Classified proteins and phosphorylation events mapped onto mTOR signaling pathway.*** *Box colors indicate protein level direction, while circle and its color indicates phosphorylation event and direction.*



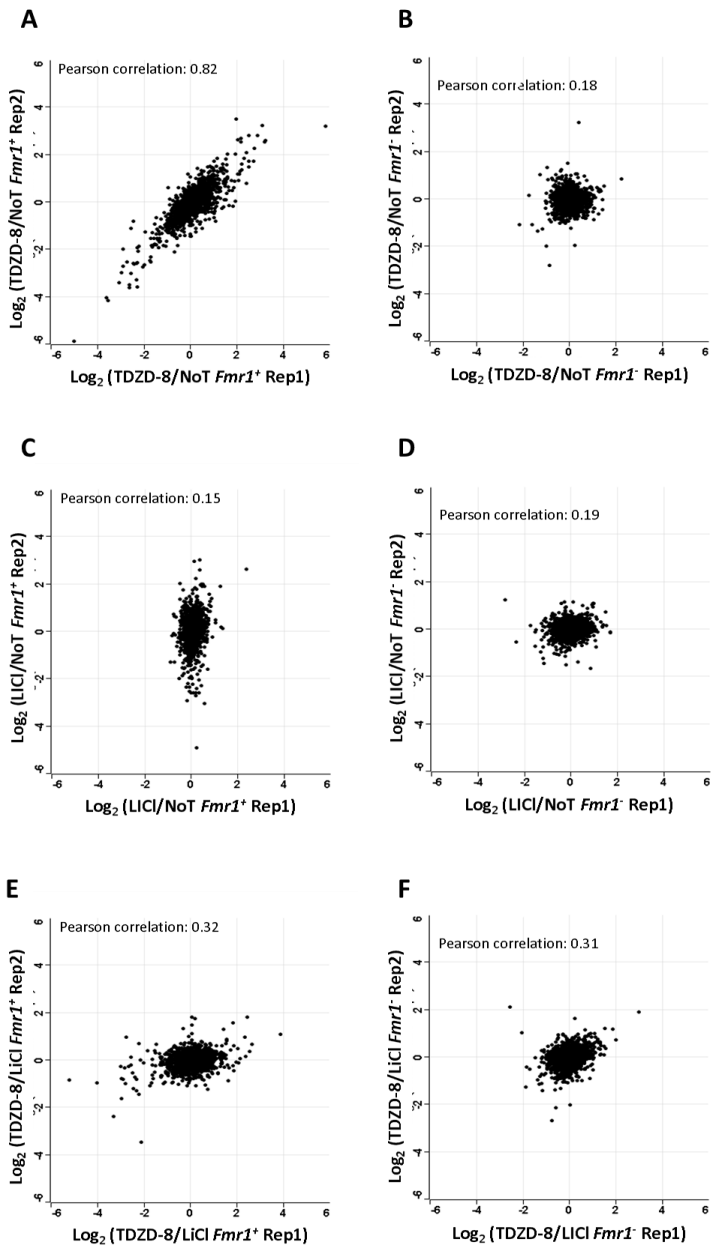

***Supplementary figure IV.3 | Classified proteins and phosphorylation events mapped onto p53 signaling pathway.*** *Box colors indicate protein level direction, while circle and its color indicates phosphorylation event and direction.*



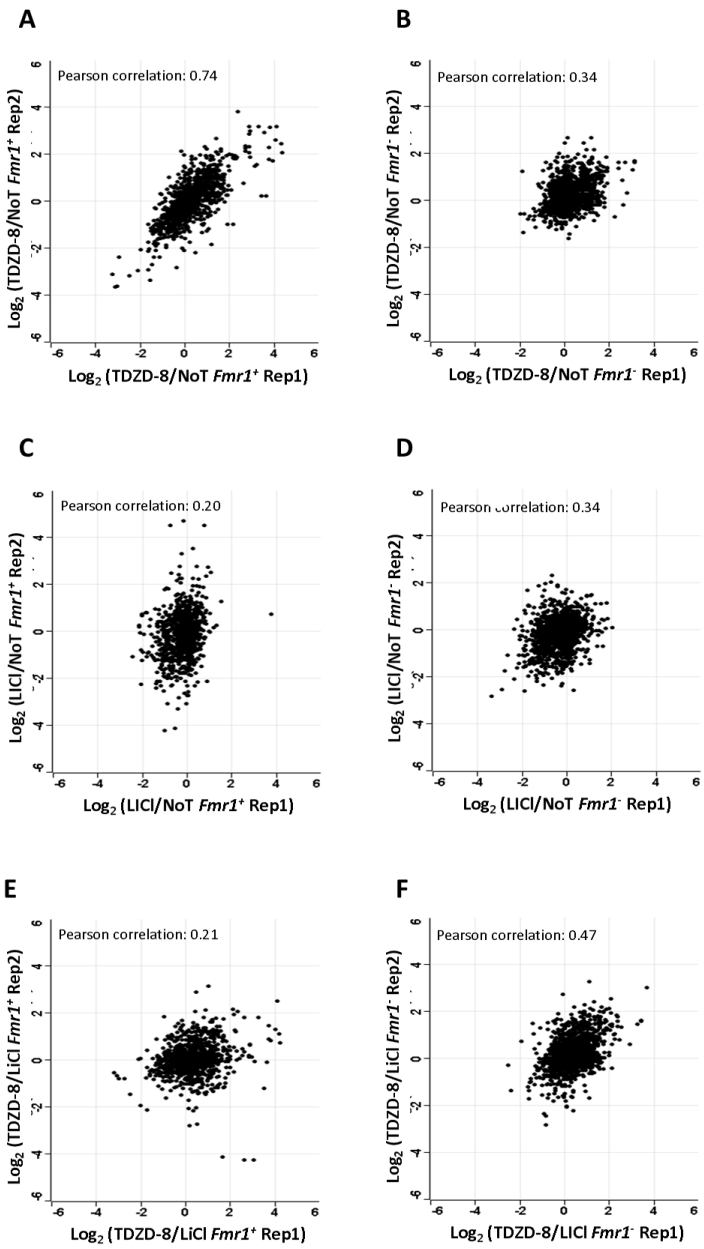

***Supplementary figure IV.4 | Classified proteins and phosphorylation events mapped onto Wnt signaling pathway.*** *Box colors indicate protein level direction, while circle and its color indicates phosphorylation event and direction.*



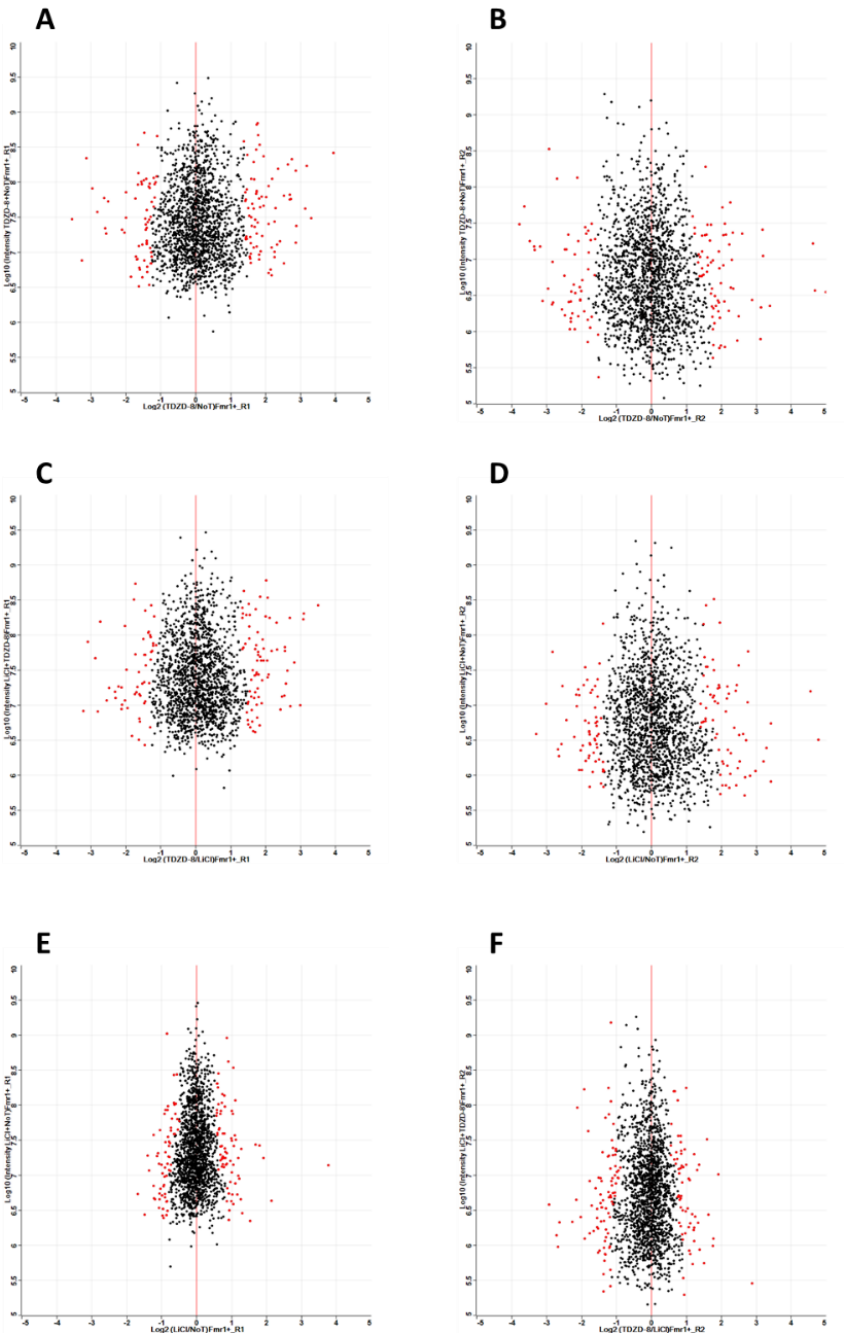

***Supplementary figure IV.5 | Classified proteins and phosphorylation events mapped onto MAPK signaling pathway.*** *Box colors indicate protein level direction, while circle and its color indicates phosphorylation event and direction.*



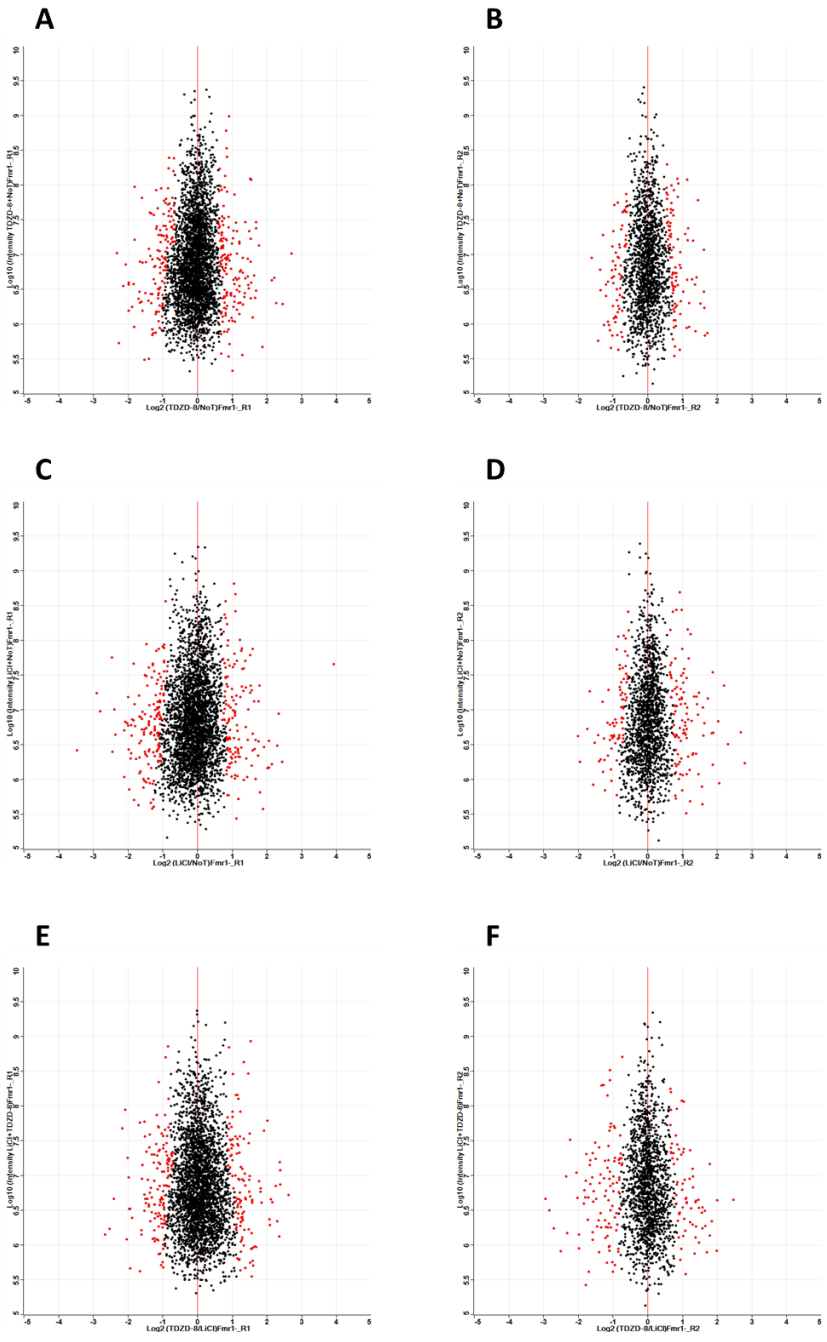

***Supplementary figure IV.6 | Correlation between biological replicates on the proteome level***. *Correlation between biological replicates in **A)** TDZD-8 treatment in Fmr1$^{+}$ MEFs; **B)** TDZD-8 treatment in Fmr1$^{-}$ MEFs; **C)** lithium treatment in Fmr1$^{+}$ MEFs; **D)** lithium treatment in Fmr1$^{-}$ MEFs; **E)** TDZD-8 versus lithium treatment in Fmr1$^{-}$ MEFs; **F)** TDZD-8 versus lithium treatment in Fmr1$^{-}$ MEFs.*



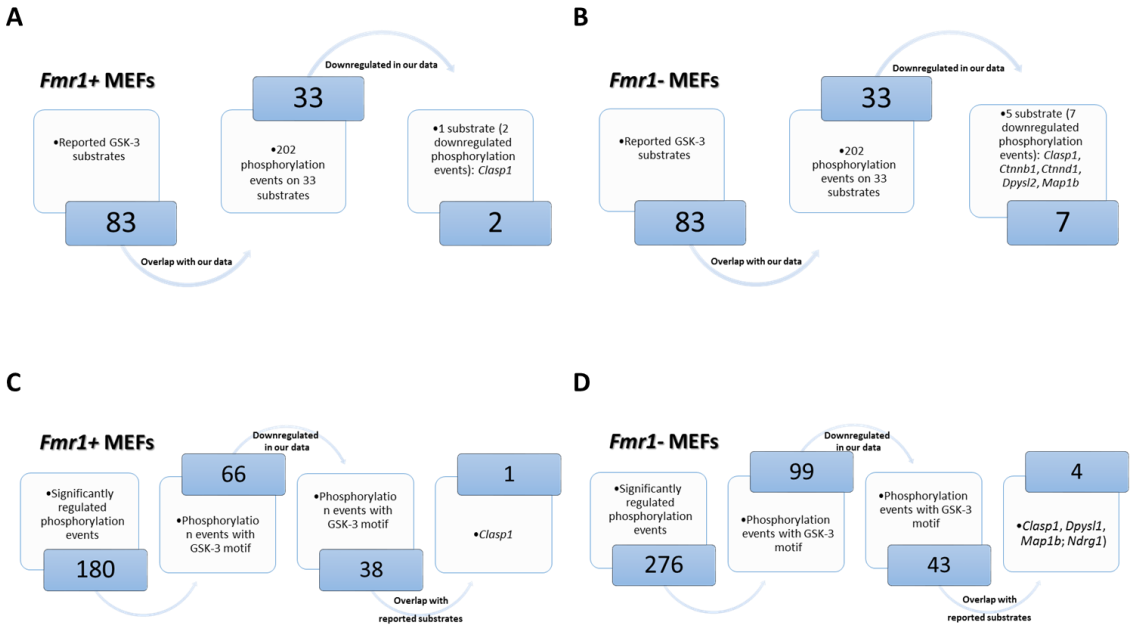

***Supplementary figure IV.7 | Correlation between biological replicates on the phosphoproteome level****. Correlation between biological replicates in* ***A)*** *TDZD-8 treatment in Fmr1⁺ MEFs;* ***B)*** *TDZD-8 treatment in Fmr1⁻ MEFs;* ***C)*** *lithium treatment in Fmr1⁺ MEFs;* ***D)*** *lithium treatment in Fmr1⁻ MEFs;* ***E)*** *TDZD-8 versus lithium treatment in Fmr1⁻ MEFs;* ***F)*** *TDZD-8 versus lithium treatment in Fmr1⁻ MEFs.*



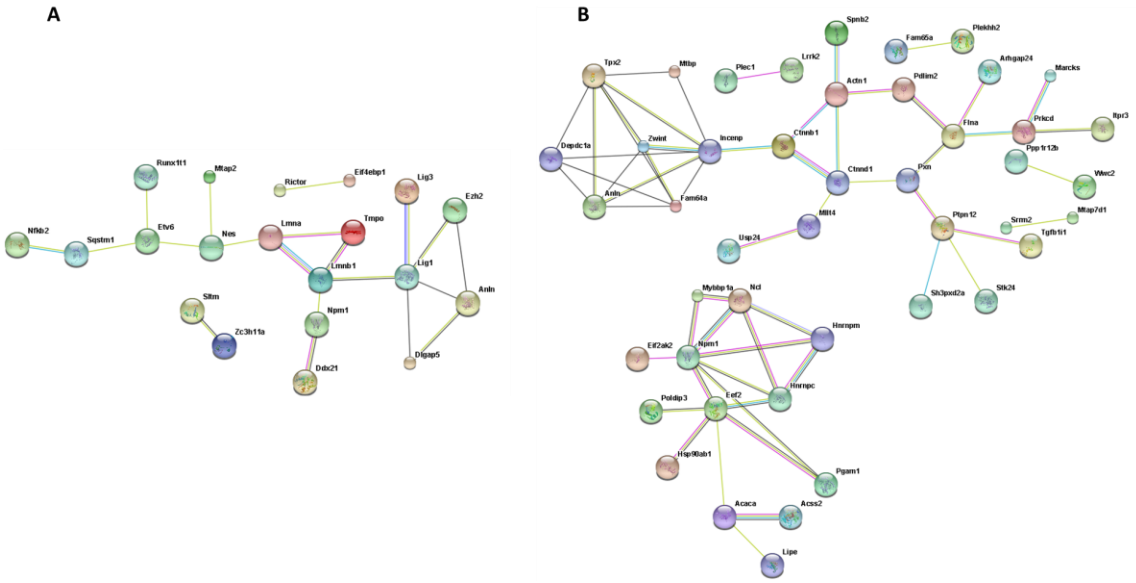

***Supplementary figure IV.8 | Distributions of quantified phosphorylation events in Fmr1⁺ MEFs. A)*** *TDZD-8 vs no treatment in biological replicate 1;* ***B)*** *TDZD-8 vs no treatment in biological replicate 2;* ***C)*** *lithium vs no treatment in biological replicate 1;* ***D)*** *lithium vs no treatment in biological replicate 1;* ***E)*** *TDZD-8 vs lithium in biological replicate 1;* ***F)*** *TDZD-8 vs no lithium in biological replicate 1;. Intensity is log10, H/L ratios log2 transformed. Red dots represent significant ($p < 0.05$) outliers.*



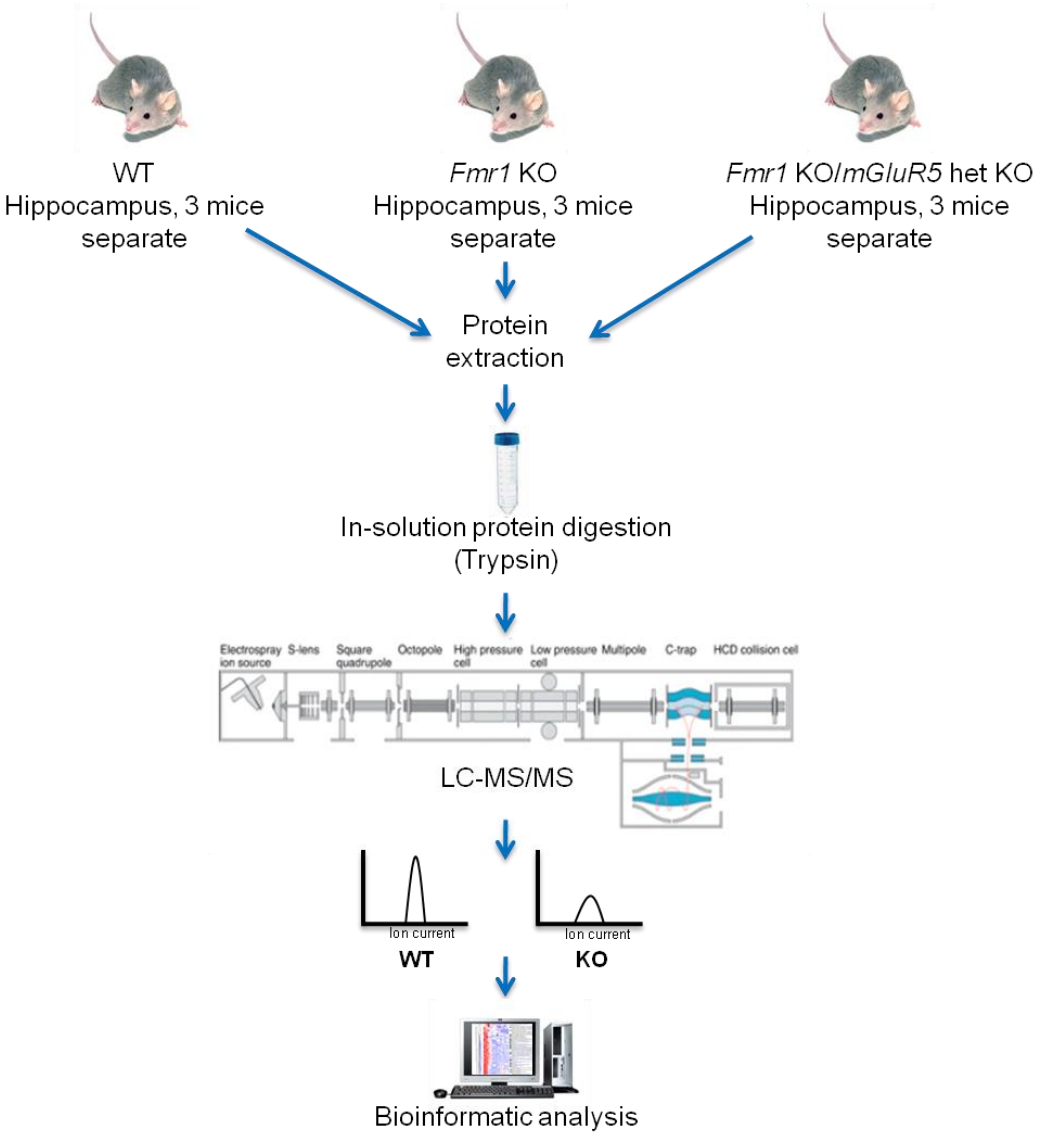

***Supplementary figure IV.9 | Distributions of quantified phosphorylation events in Fmr1⁻ MEFs. A)*** *TDZD-8 vs no treatment in biological replicate 1;* ***B)*** *TDZD-8 vs no treatment in biological replicate 2;* ***C)*** *lithium vs no treatment in biological replicate 1;* ***D)*** *lithium vs no treatment in biological replicate 1;* ***E)*** *TDZD-8 vs lithium in biological replicate 1;* ***F)*** *TDZD-8 vs no lithium in biological replicate 1;. Intensity is log10, H/L ratios log2 transformed. Red dots represent significant ($p < 0.05$) outliers.*



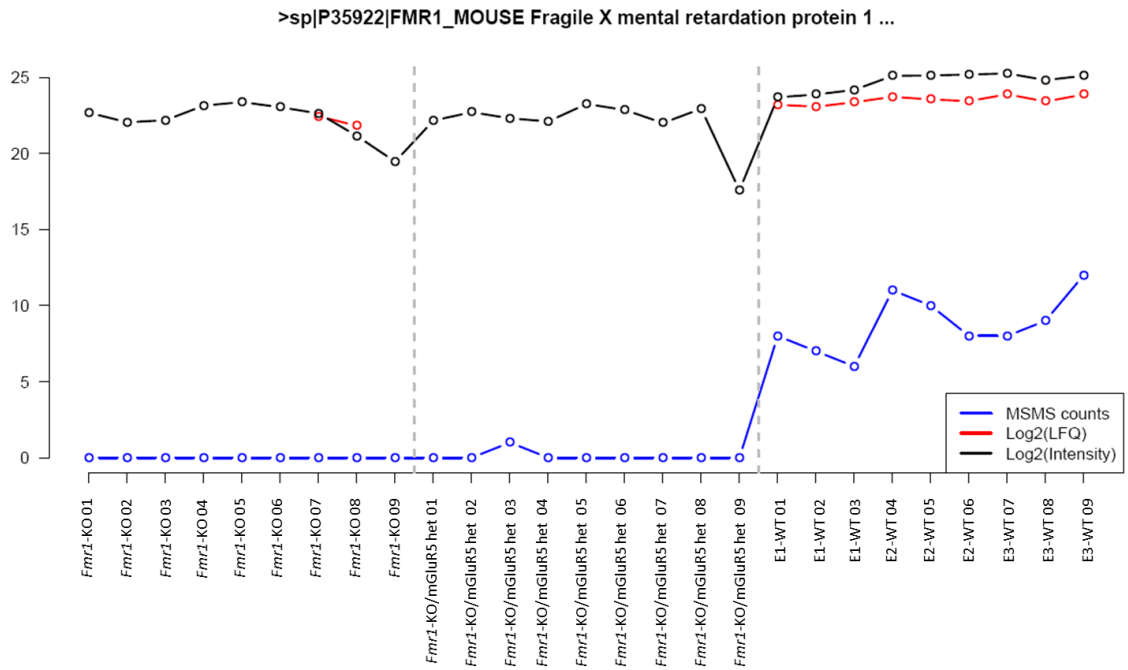

***Supplementary figure IV.10 | Overview of overlap with known substrates and GSK motif in our phosphoproteome data upon lithium treatment. A)*** *Overlap with reported (known and proposed) substrates in Fmr1$^{+}$ MEFs;* ***B)*** *Overlap with reported (known and proposed) substrates in Fmr1$^{-}$ MEFs;* ***C)*** *Number of phosphorylation proteins with GSK motif in Fmr1$^{+}$ MEFs;* ***D)*** *Number of phosphorylation proteins with GSK motif in Fmr1$^{+}$ MEFs.*



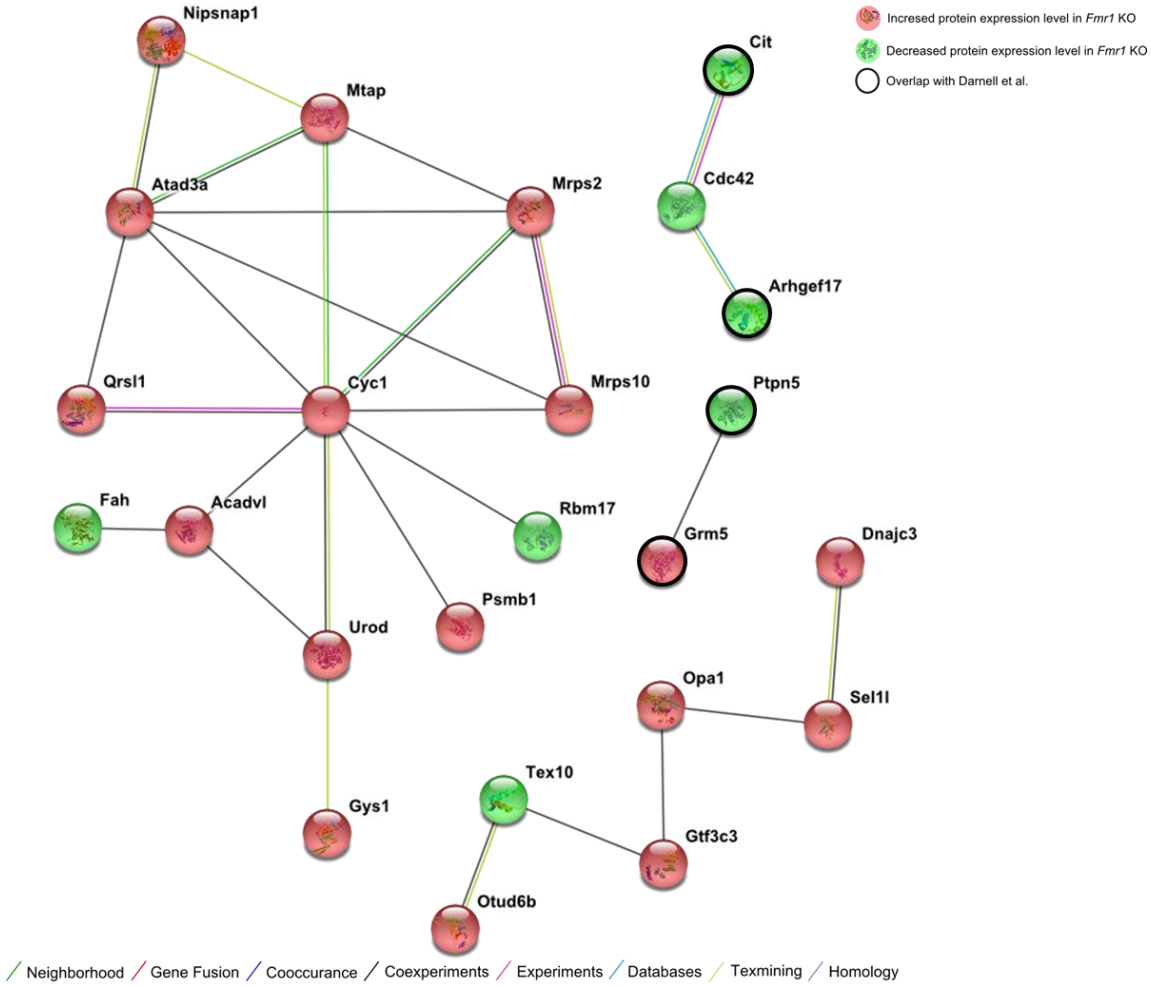

***Supplementary figure IV.11 | Known and predicted protein interactions of detected phosphoproteins upon lithium treatment. A)*** *proteins with decreased phosphorylation in Fmr1$^{+}$ cell line;* ***B)*** *proteins with decreased phosphorylation in Fmr1$^{-}$ cell line.*



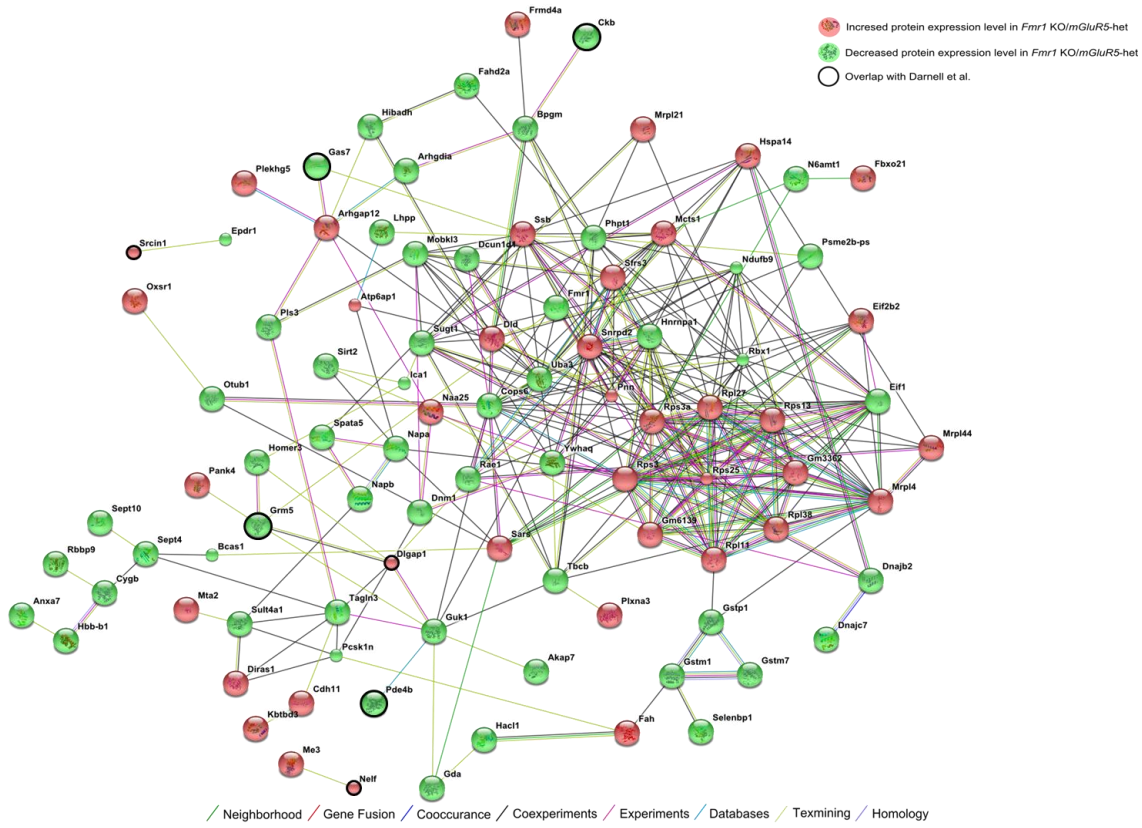

***Supplementary figure IV.12 | Quantitative proteomic workflow.*** *Proteins were extracted from isolated hippocampi of WT, Fmr1 KO and Fmr1 KO/mGluR5 het KO mice and digested with Trypsine. The resulting peptides were analyzed by LC-MS/MS after which bioinformatic analysis was performed.*




***Supplementary figure IV.13 | MS/MS counts, label-free intensity and intensity of FMRP.*** *Intensity is observed across all genotypes in all samples (black line), while label-free (LFQ) intensity is present in all WTs and two Fmr1-Kos (red line). In order to get value for the LFQ intensity, 2 peptides from the same proteins are needed. LFQ intensity in Fmr1-KOs (as well as intensity in both KOs) can be explained via manual observation of two peptides which are giving LFQ intensity. These peptides are not unique for FMRP protein, and moreover no MS/MS counts were observed in these Fmr1-KO samples (blue line). We enabled match between run option during the processing of the data which is responsible for this artefact.*




***Supplementary figure IV.14 | STRING analysis****. Network of significantly changing proteins from pairwise analysis of Fmr1-KO and Fmr1-KO/mGluR5 het genotype. Red color indicates increased protein expression, and green decreased protein expression in Fmr1-KO, respectively. Black circle means that this proteins are overlapping with proteins from Darnell et al. dataset.*




***Supplementary figure IV.15 | STRING analysis***. *Network of significantly changing proteins from pairwise analysis of WT and Fmr1-KO/mGluR5 het genotype. Red color indicates increased protein expression, and green decreased protein expression in Fmr1-KO, respectively. Black circle means that this proteins are overlapping with proteins from Darnell et al. dataset.*