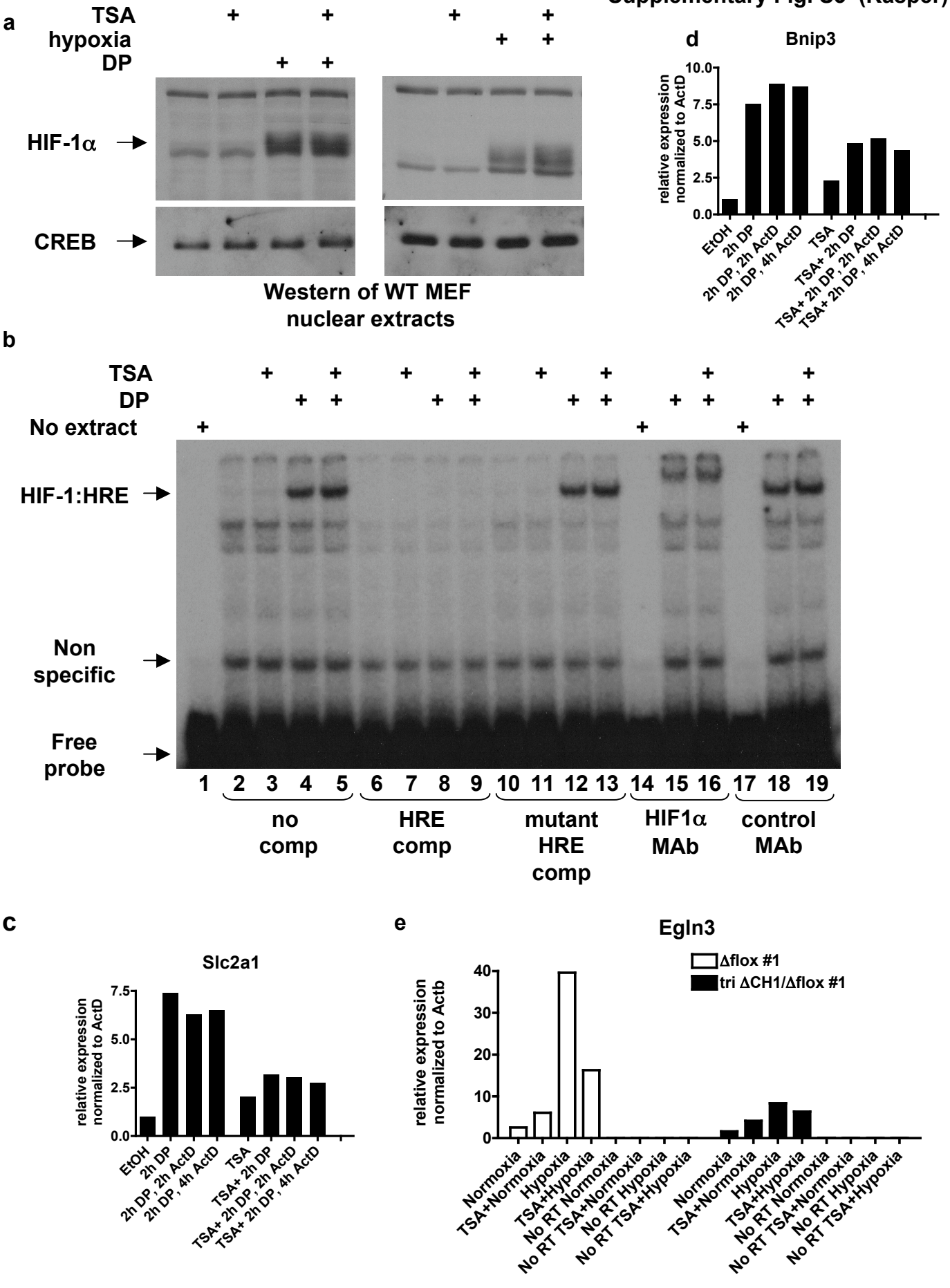
Supplementary Fig. S3 (Kasper)



Supplementary Fig. S3. TSA treatment does not affect HIF-1α protein stability, nuclear entry, DNA binding, or stability of HIF-1α-responsive gene mRNA**. a**, Western blot of HIF-1α and CREB (as a control) in nuclear extracts showing equivalent stabilization of HIF-1α protein in wild type MEFs, with or without 30 minute pretreatment with TSA, treated for 5 hours with DP or 6 hours under hypoxia. **b**, Electrophoretic mobility shift assay (EMSA) of nuclear extracts from WT MEFs treated with ethanol vehicle (lanes 2, 4, 6, 8, 10, 12, 15 and 18) or TSA (lanes 3, 5, 7, 9, 11, 13, 16 and 19) for 30 minutes before treatment with DP (lanes 4, 5, 8, 9, 15, 16, 18 and 19) or ethanol vehicle (lanes 2, 3, 6, 7, 10 and 11) for an additional 5 hours. Lanes 1, 14 and 17 do not contain nuclear extract. A radiolabeled double-stranded oligonucleotide probe containing an HRE from the promoter of the mouse *Pfkfb3* gene was used along with 1 μg of poly-dIdC per reaction. Unlabeled probe was used as a competitor (HRE comp) as was a mutated oligo that cannot bind HIF (mutant HRE comp). HIF-1α monoclonal antibody could shift the complex (HIF1α MAb) while a P/CAF monoclonal antibody could not (control MAb). Specific (HIF-1:HRE) and non-specific complexes are indicated. **c,d,** qRT-PCR analysis of *Slc2a1* (**c**) and *Bnip3* (**d**) mRNA in wild type MEFs normalized to β-actin mRNA. Cells were treated with ethanol vehicle or TSA; after 30 minutes, ethanol vehicle (EtOH) or DP was added, then after 2 hours, Actinomycin D (ActD) or EtOH was added. Cells were harvested for RNA at the time of ActD addition (2h DP), and 2 hours or 4 hours after ActD was added (2h DP, 2h ActD or 2hDP, 4h ActD respectively). **e,** qRT-PCR analysis of cDNA reverse transcribed from primary unspliced *Egln3* RNA transcripts, normalized to β-actin unspliced RNA, using PCR primer pairs that span an exon-intron boundary. Medium on Δflox #1 and tri-ΔCH1/Δflox #1 MEFs was removed at start of experiment and replaced with medium that had been equilibrated overnight in a hypoxic or normoxic incubator and to which TSA or EtOH vehicle was added immediately before medium change. Cells were then incubated for 3h under hypoxia or normoxia followed by cell harvest and RNA extraction. RNA was treated with DNase before reverse transcription. As a check for genomic DNA contamination, samples with no reverse transcriptase added (No RT) were analyzed simultaneously.