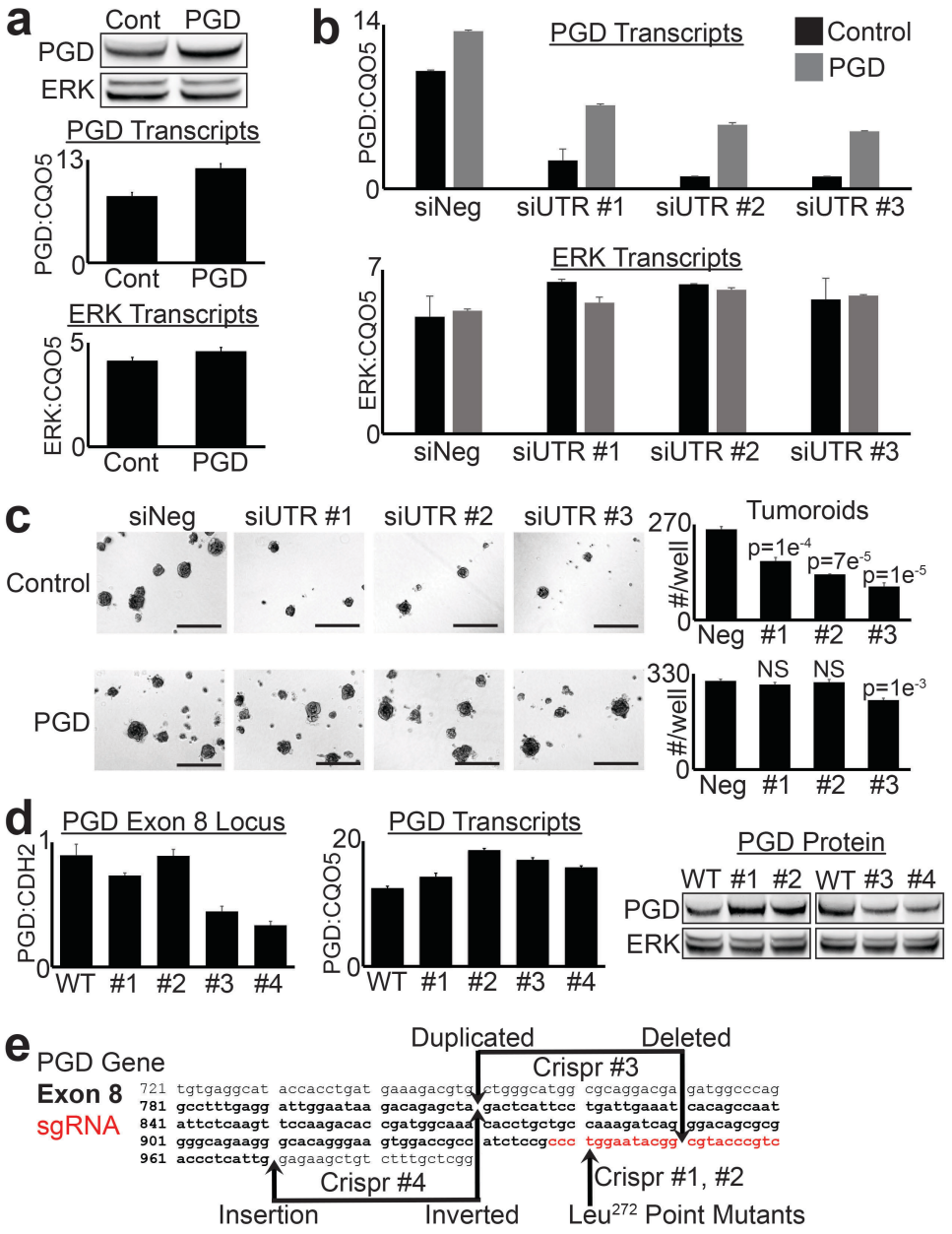
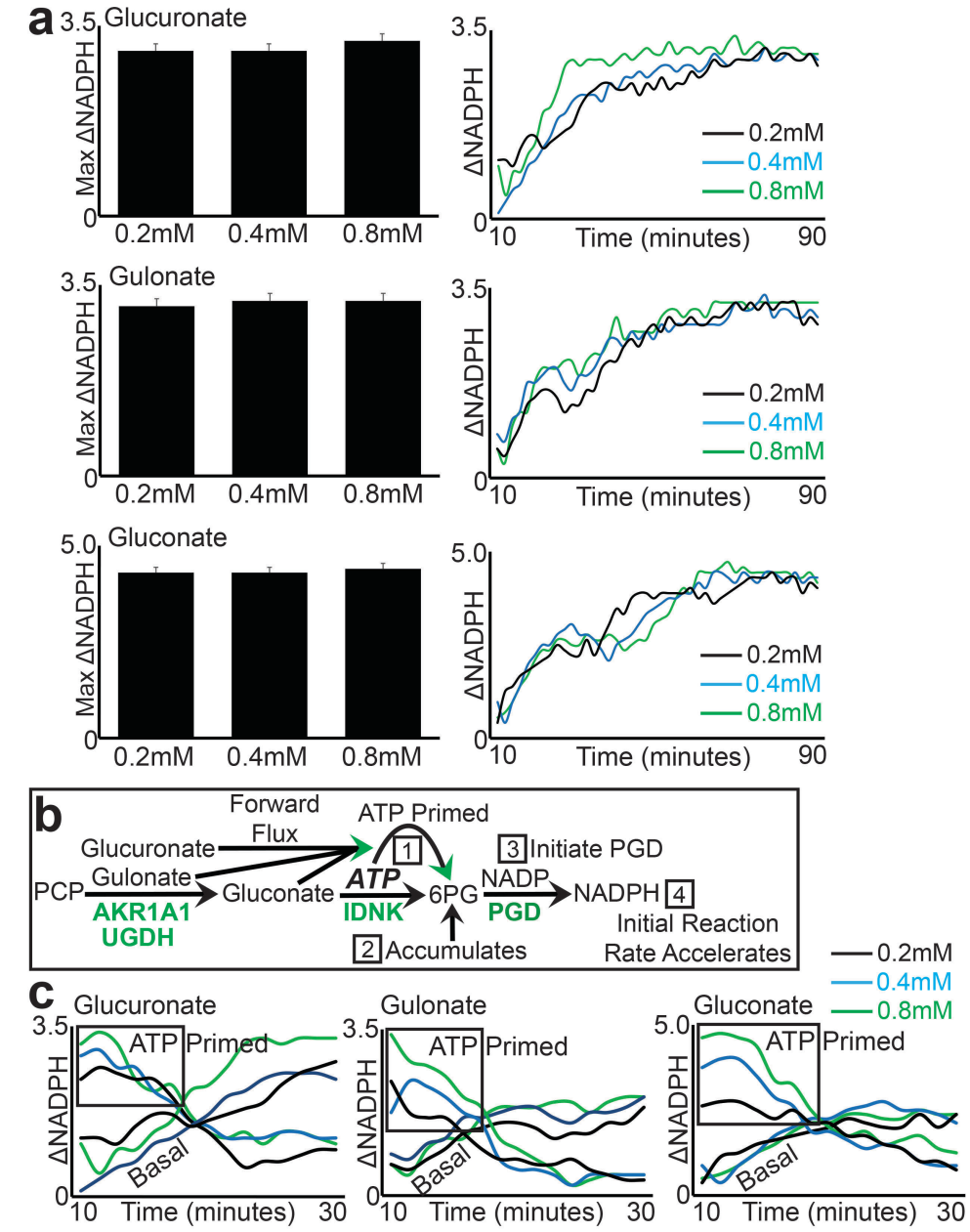
Supplementary Figure 1



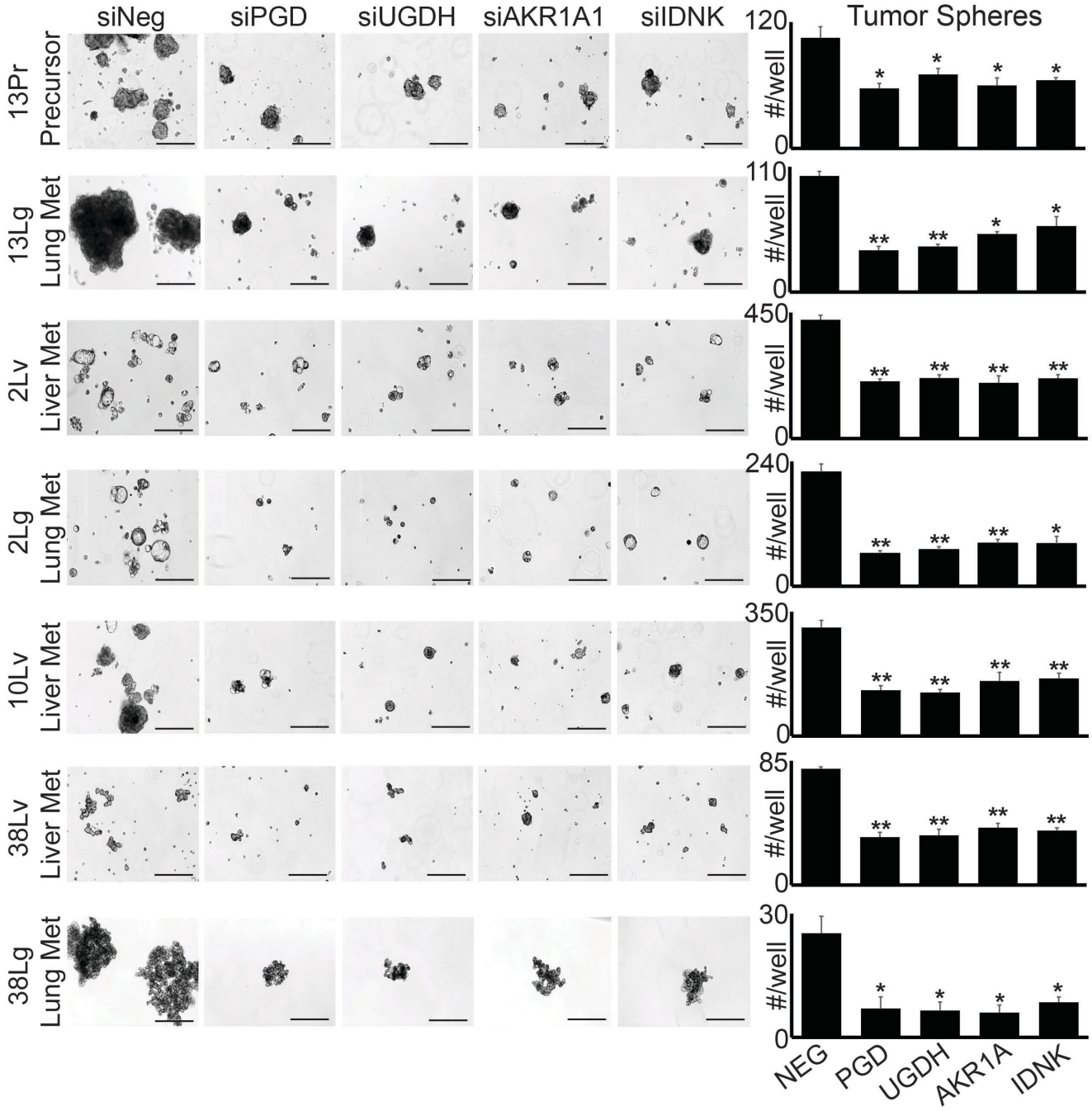
Supplementary Figure 1: Rescue of PGD knockdown and characterization of PGD Crispr clones. (**a**) Exogenous PGD under control of an EF1a promoter was modestly expressed in the 38Lg lung metastasis subclone, as shown by western blots and qRT-PCR (MOI: 5; Sigma TRC3 ORF). Controls (Cont) are 38Lg cells similarly transduced with GFP under the control of an EF1a promoter. (**b**) Exogenous *PGD* expression was retained during knockdown of endogenous *PGD* with siRNAs targeted to the 3'-UTR. (**c**) Exogenous PGD rescued 3D tumoroid growth during RNAi knockdown experiments. Statistical significance was calculated using 2-tailed t-tests. (**d**) Real-time PCR on genomic DNA (left panel) using primers flanking PGD exon 8 revealed a ~50% reduction in amplification signals from the loss-of-function Crispr clones (#3, #4), reflecting heterozygous rearrangement events that block primer extension. Although expression of PGD mRNA transcripts were similar (middle panel), PGD protein expression was increased in gain-of-function clones (#1, #2) and decreased in loss-of-function clones (#3, #4) by western blots (right panel). (**e**) The western blot findings may reflect the intrinsic protein stabilities of the mutated alleles, since clones #1 and #2 shared identical point mutations while clones #3 and #4 acquired complex rearrangements within and around the sgRNA sequence (red fonts).

Supplementary Figure 2



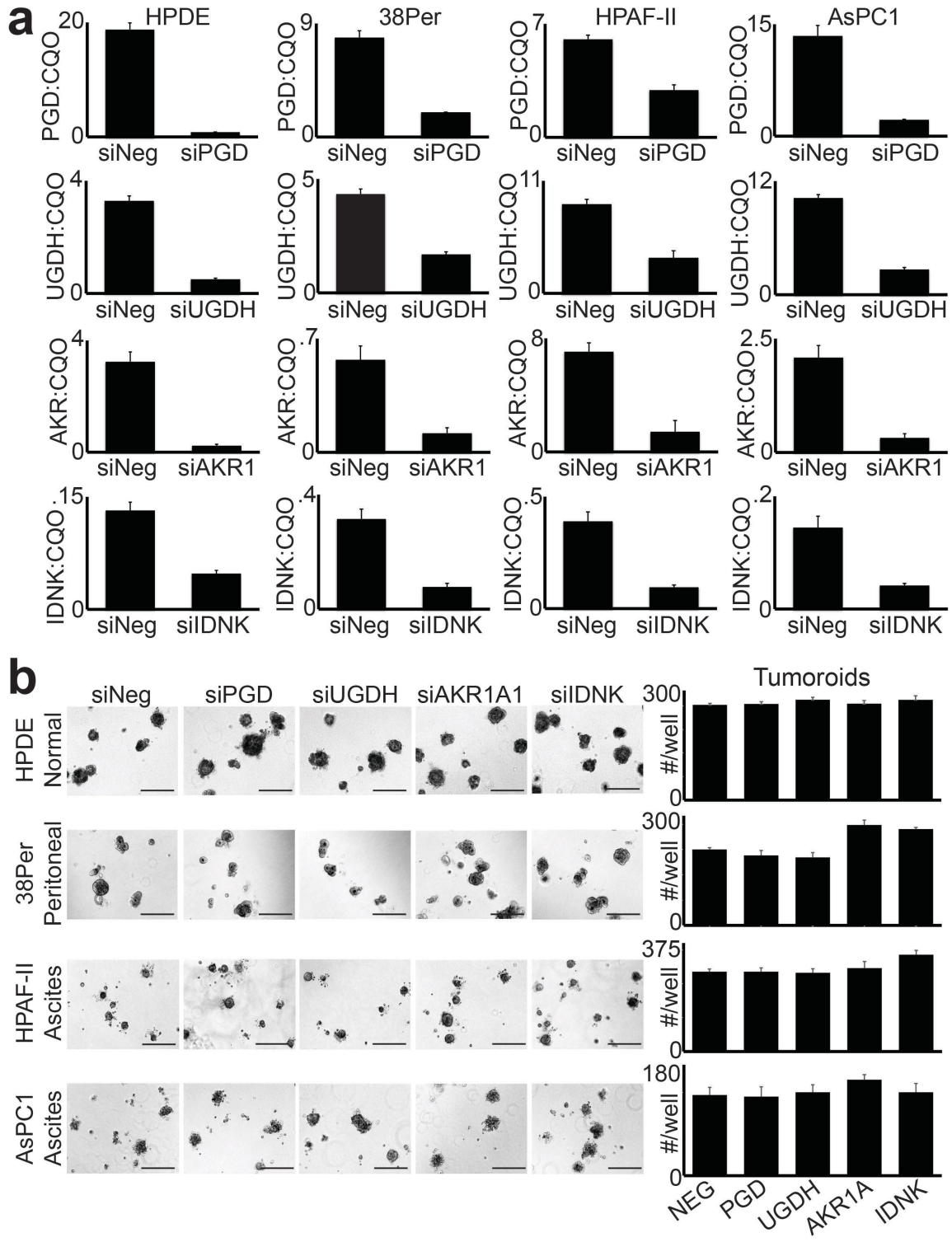
Supplementary Figure 2: Flux of pentose substrates through the PCP. (**a**) Increasing amounts of the indicated PCP substrates were used for PGD enzyme assays in extracts from the 38Lg lung metastasis subclone. All reactions used 0.2mM of NADP. NADPH production was stimulated to comparable rates across all substrate concentrations, suggesting that PGD catalytic activity drives forward flux of pentose substrates into the PCP (confirmed in Fig. 2e). (**b**) Schematic illustrating the experimental design of data presented in Fig. 2d and Supp. Fig. 2c. Pre-incubation of cell extracts with PCP substrates and ATP ("ATP Priming": step 1) prior to initiating the PGD reaction draws substrate flux forward through the IDNK step of the pathway, allowing 6PG to accumulate (step 2). After 6PG accumulates to high levels, initiating the PGD reaction with NADP (step 3) greatly accelerates PGD-driven NADPH production rates (step 4). (**c**) Pre-incubating extracts as described above ("ATP Primed") followed by initiating the PGD reaction under ice cold temperatures prolongs the accelerated phase of the reaction (boxed areas). This reveals that increasing substrate concentrations enhances PGD catalytic activity during the accelerated phase (compare with the basal rates below the boxes), consistent with proportional conversion of each pentose into 6PG as predicted by the PCP model.

Supplementary Figure 3



Supplementary Figure 3: PCP enzymes are required for 3D tumorigenesis (tumorsphere assays). Metastatic subclones were transfected with the indicated siRNAs, trypsinized into single cells, and plated into low attachment plates with stem cell media (DMEM:F12 supplemented with B-27, N-2, insulin, transferrin, bFGF, and EGF) as described previously (ref. 24). Knockdown of PCP enzymes impaired tumorsphere growth across all subclones with similar efficacy as PGD knockdown, relative to controls (siNeg). Statistical significance was calculated using 2-tailed t-tests (*$p<0.05$; **$p\leq0.001$). Scale bars: 400μm.

Supplementary Figure 4



Supplementary Figure 4: PCP enzymes are dispensable for 3D tumorigenesis in PGD-independent control cells. (**a**) Control cells (HPDE, 38Per, HPAF-II, AsPC1) were transfected with control siRNAs (siNeg) and siRNAs against *PGD* and PCP enzymes as indicated. RNA was isolated four days later and each gene amplified (qRT-PCR) to ensure adequate knockdown in control cells. Transcripts were normalized to *CQO5*, since levels of that transcript remained constant across all conditions. (**b**) Cells were transfected as above and plated into suspension matrigel assays. RNAi knockdown did not impair the ability of these cells to form tumoroids in 3D.