Supplemental Figure Legends:

**Supplemental Figure 1. Compound from parental class suppresses SIX1-EYA2 transcriptional activity.** Transcriptional activity of a MEF3 promoter luciferase reporter in the presence of EYA2 + an empty vector, EYA2 + SIX1 WT, EYA2 + SIX1 V17E mutant, and EYA2 + SIX1 WT + parental compound in MCF7 cells.

**Supplemental Figure 2. Synthesis of compound 8430. a.** The synthesis of NCGC00378430 was initiated by the nucleophilic substitution of 2-methoxy-5-nitrobenzenesulfonyl chloride with morpholine followed by a palladium-on-carbon catalyzed hydrogenation of the nitro group to the aniline. **b.** This aniline was then subjected to HATU-mediated amide coupling with commercially available 3-(1*H*-pyrrol-1-yl)benzoic acid to provide NCGC00378430 (*N*-(4-methoxy-3-(morpholinosulfonyl)phenyl)-3-(1*H*-pyrrol-1-yl)benzamide).

**Supplemental Figure 3. Endogenous SIX1 and EYA2 expression in breast cancer cells.** Representative WB (of n=3) demonstrates endogenous protein levels of SIX1 and EYA2 in HMLER, MCF7, T47D and MDA-MB-231 cells.

**Supplemental Figure 4. 8430 influences SIX1-mediated transcription. a.** Volcano plot demonstrates differential gene expression patterns when comparing across Ctrl+Veh, SIX1+Veh and SIX1+8430 conditions. A filter of adjusted *p* value<0.1 was applied to RNA sequencing data. All genes that passed this filter are plotted. Those genes highlighted in red (up) /blue (down) have an absolute adjusted fold change >2. **b.** Heatmap demonstrates hierarchical clustering of z-score-converted expression values of significantly differentially expressed genes (p-adj < 0.1 and absolute adjusted fold change > 2) between the WT and SIX1 OE conditions, and three predominant and distinct clusters of differential gene expression are revealed. **c.** Gene ontology (GO) enrichment analysis was performed using genes oppositely altered by SIX1 OE and 8430 treatment. Top 15 most enriched pathways are plotted (ranked by *p* value). Pathways directly relevant to development are labeled in red while others are labeled in blue. *P* value is highlighted in green, while enrichment scores are listed on top of each bar.

**Supplemental Figure 5. 8430 is well-tolerated in mice. a.** Pharmacokinetics of 8430 in mice following a 20mg/kg IV bolus dose. PK parameters were calculated via a 2-compartment model using the average measured values for mice at each time point. Concentration of 8430 in plasma was plotted across time, error bars are standard deviations. **b.** Comparison of 8430 plasma levels at 1, 2, and 4 hours post-dosing via either the IV or IP route. Concentration of 8430 in plasma was plotted across time, error bars are standard deviations. **c.** Weight measurement of mice treated by various doses of 8430. Mice were treated by 8430 at 0 (vehicle), 15, 20 or 25mg/kg for three weeks (delivered to 4th mammary fat pad every other day) and percentage of weight gain is plotted. Error bars are standard deviations, and statistical significance was evaluated by one-way ANOVA followed by Tukey post adjustment, *p* value is listed with 4 decimal places. **d.** Photomicrograph demonstrates examples of histological changes observed in livers of animal treated by 8430. *Left,*a single, small (<0.5mm diameter) focus of hepatic necrosis, with replacement fibrosis was observed in one animaltreated by 15mg/kg 8430. Few individualized necrotic hepatocytes are present along the margin of fibrosis. Low numbers of lymphocytes infiltrate the fibrous tissue. 20x magnification. *Middle,*another example of typical histological lesions observed in the livers of animals treated by 20mg/kg 8430. There is rare, individual single cell hepatocyte necrosis (arrows), with infiltration of the hepatic parenchyma by few lymphocytes, neutrophils, and macrophages. 20x magnification. *Right,*there is a single, sub-capsular nodule of coagulative necrosis of hepatocytes (arrow heads), with mild reactive fibrosis of the overlying hepatic capsule (arrow) in one animal treated by 25mg/kg 8430. 10x magnification.

**Supplemental Figure 6. 8430 reduces metastases in mice.** At the end of animal study, primary tumors were harvested and distant metastases were examined. **a.** Representative image demonstrates that turbo RFP-tagged MCF7-SIX1 tumor cells (in primary tumors) show strong red signal under room light, enabling the macroscopic detection of disseminated tumor cells. **b.** Representative images demonstrate that macroscopic lymph node metastases (arrow) were detected in the vehicle group (3 out of 12) but not in 8430 treated condition.