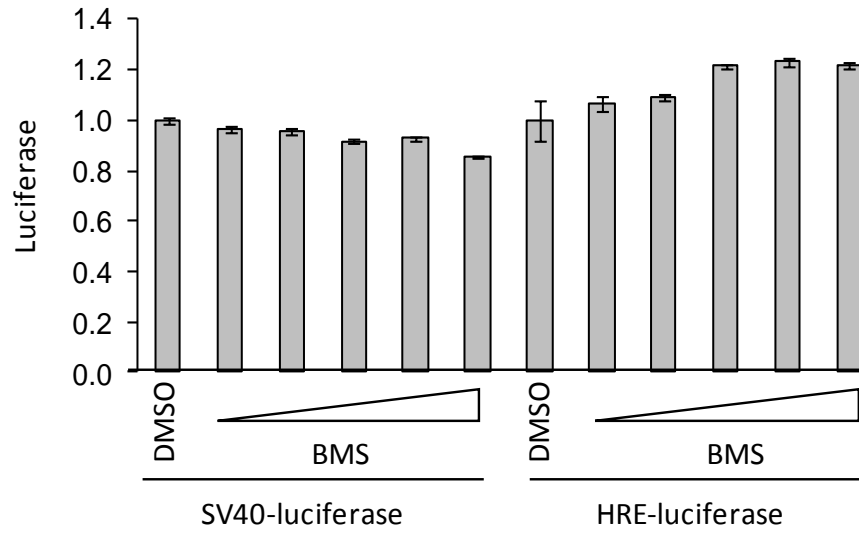
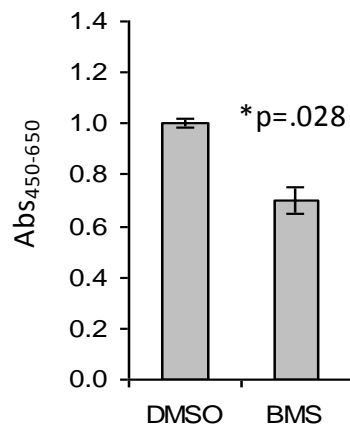


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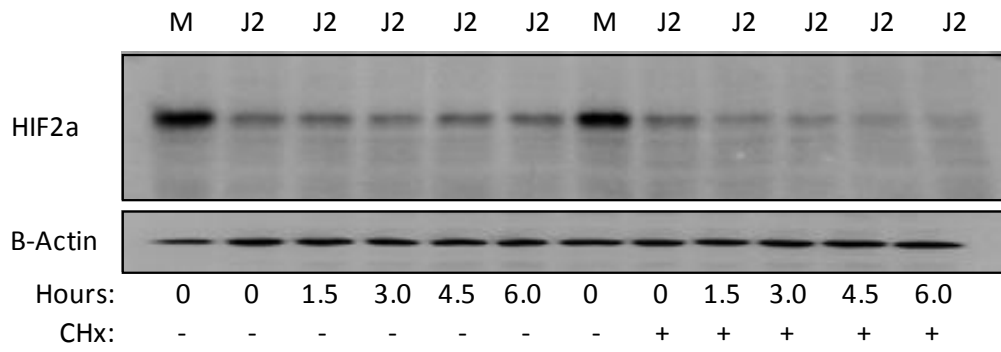


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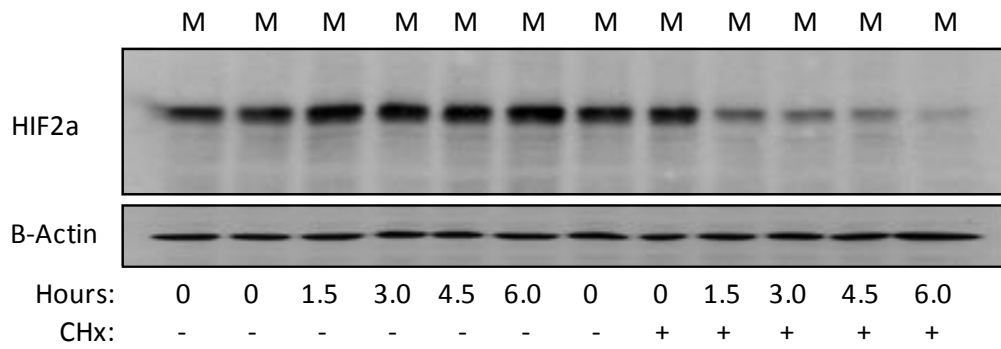


SUPPLEMENTARY FIGURE 1

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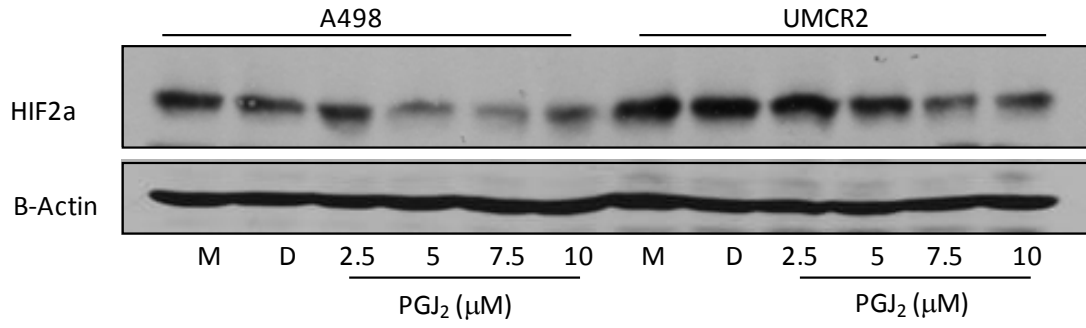


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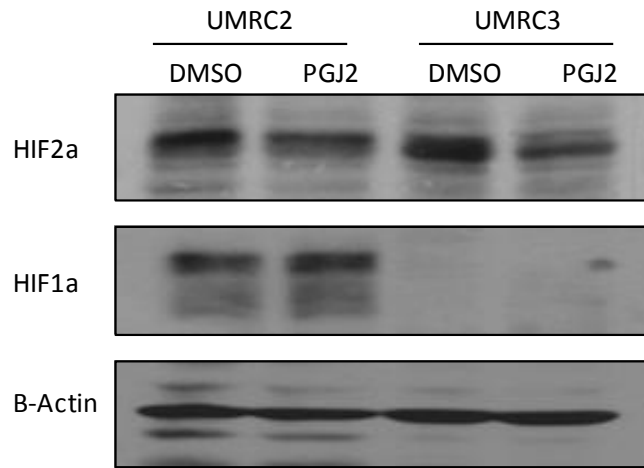


SUPPLEMENTARY FIGURE 2

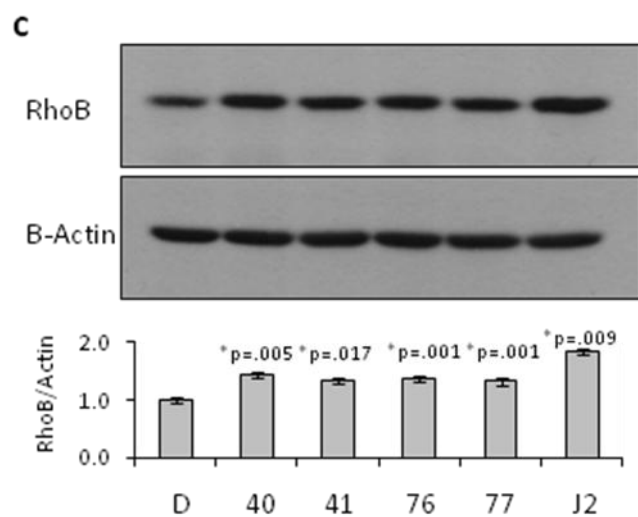
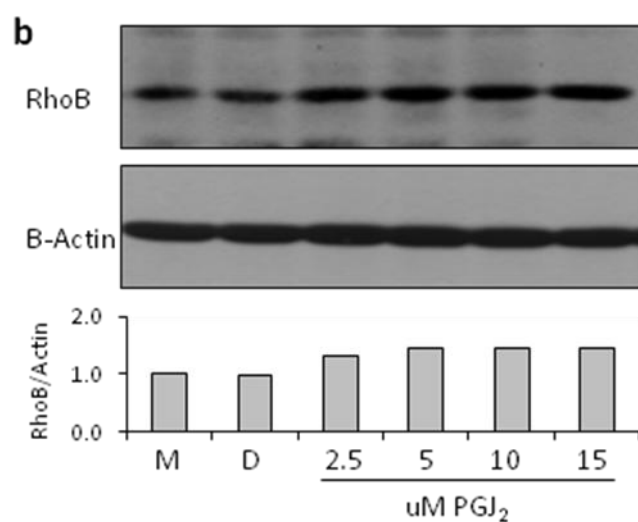
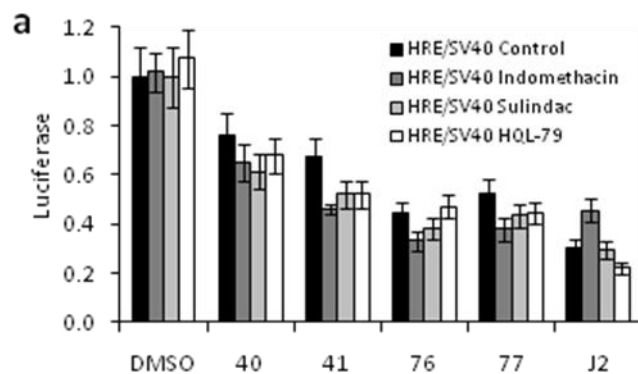
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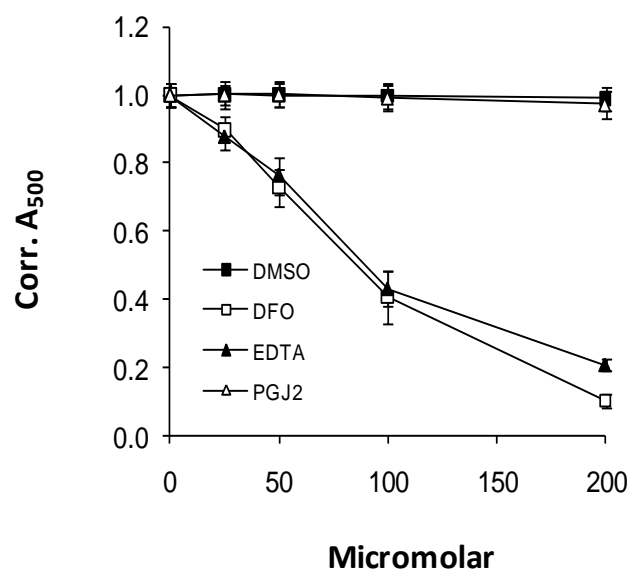
b



SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4



SUPPLEMENTARY FIGURE 5

Supplementary Figure 1. NFkB inhibition does not affect activity of HIF2a. (a) Normalized luciferase activity from stable SV40- and HRE-luciferase expressing 786-O cells, treated for 24 hours with the IKK Inhibitor BMS-345541, at the indicated concentrations, relative to DMSO-only treated control. (b) TransAM NFkB ELISA (Active Motif, cat.# 100627) demonstrating that basal NFkB activity is suppressed in 786-O cells when 5 μ M BMS-345541 is applied for 24 hours. Experiments were performed in triplicate. Error bars represent standard error of the mean (SEM).

Supplementary Figure 2. PGJ₂ does not affect HIF-2a stability as measured by blocking *de novo* protein synthesis with cycloheximide. (a) *Effect of PGJ₂ on HIF-2a protein stability.* 786-O cells were treated with PGJ₂ for 2 days, as described for Figure 1b. *De novo* protein synthesis was then halted by the addition of 10 μ g/mL cycloheximide (CHx) and time points taken every 1.5 hours for 6 hours. Lanes 1-6, control for HIF-2a expression without addition of cycloheximide (CHx) (lane 1, DMSO-only; lanes 3-6, 10 μ M PGJ₂); lanes 7-12, HIF2a expression following the addition of CHx (lane 7, DMSO-only, lanes 8-12, 10 μ M PGJ₂ harvested at 0, 1.5, 3, 4.5 and 6 hours following the addition of CHx). B-Actin is shown for loading control. (b) *HIF2a protein stability in 786-O cells treated with DMSO-only.* Identical experiment as described above without addition of PGJ₂.

Supplementary Figure 3. Inhibition of HIF2a expression by PGJ₂ is not a cell specific phenomenon. (a) *PGJ₂ inhibits HIF2a in several cell lines.* The VHL-null human renal cell carcinoma cell lines A498 and UMRC2 were treated with increasing

doses of PGJ₂ as indicated. HIF2a expression was analyzed by Western blot. B-actin loading control is shown. (b) *PGJ2 affects preferentially HIF2a but not HIF1a*. VHL-deficient UMRC2 and UMRC3 lines were treated with DMSO or 15 μM PGJ₂. HIF1a, HIF2a and B-actin (loading control) expression was analyzed by Western blot.

Supplementary Figure 4. Small molecule HIF2a inhibitors do not appear to increase endogenous PGJ₂ levels. (a) *Cyclooxygenase 1/2 inhibition does not prevent the effect of PGJ2 or HIF2a inhibitors on HIF2a activity*. Luciferase assay showing the effect of compound and PGJ₂ treatment of 786-O derived HRE- over SV40 (control) luciferase reporter lines in the presence of 10 μM Sulindac, 10 μM Indomethacin (COX-1/2 inhibitors), or 50 μM HQL-79 (a PGD Synthase Inhibitor). (b) *PGJ2 upregulates the expression of the PPAR gamma target protein RhoB*. Western blot showing the effect of PGJ₂ on RhoB protein expression. 786-O cells were treated with the indicated concentrations of PGJ₂ for two days prior to harvesting. B-actin is shown for loading control. Densitometry of RhoB/B-actin expression for this specific experiment is shown in the lower panel. (c) *HIF2a inhibitors upregulate the expression of RhoB*. Western blot showing the effect of PGJ₂ and all compounds on RhoB protein expression. 786-O cells were treated for two days with 40 μM 40, 30 μM 41, 10 μM 76, 5 μM 77 or 10 μM PGJ₂. B-actin is shown for loading control. Densitometry of RhoB/B-actin averaged from three independent Western blots is shown on bottom. Error bars represent Standard Error of the Mean (SEM).

Supplementary Figure 5. PGJ₂ is not an iron chelator. Displacement of iron from ethyl-3,4-dihydroxybenzoate (EDHB) complex was measured as a decrease in absorbance at 500 nm in the presence of PGJ₂, EDTA or DFO.

Supplementary Table 1. Signature of HIF2a inhibitors used for Connectivity

Mapping.

<u>40 up</u>	<u>41 up</u>	<u>76 up</u>	<u>77 up</u>	<u>40 down</u>	<u>41 down</u>	<u>76 down</u>	<u>77 up</u>
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