Supplemental Figure 1 Biliary damage assessed by H&E in $Mdr2^{-/-}$ mice treated with or without H1HR and H2HR antagonists. In WT mice treated with either the H1HR or H2HR antagonist there is no change in hepatic architecture or damage when compared to WT treated with saline. In $Mdr2^{-/-}$ mice treated with saline there is increased inflammation and necrosis when compared to WT mice. However, hepatic inflammation and necrosis are ameliorated in $Mdr2^{-/-}$ mice treated with the H1HR antagonist, the H2HR antagonist or both. Images are 20X magnification.

Supplemental Figure 2 Small and large biliary proliferation and IBDM was quantified following Ki-67 or CK-19 staining in $Mdr2^{-/-}$ mice. In $Mdr2^{-/-}$ mice treated with saline, both small and large cholangiocytes proliferate, as demonstrated by semi-quantification of Ki-67 staining when compared to WT (A); however, small cholangiocyte proliferation is decreased following treatment with the H1HR antagonist, whereas large cholangiocyte proliferation is reduced in $Mdr2^{-/-}$ mice treated with the H2HR antagonist, when compared to saline treated $Mdr2^{-/-}$ mice. $Mdr2^{-/-}$ mice treated with both the H1HR and H2HR antagonists have a decrease in both small and large biliary proliferation when compared to saline treated mice (A). Similar to proliferation, both small and large IBDM is enhanced in $Mdr2^{-/-}$ mice treated with saline compared to WT; however, large IBDM is significantly greater than small IBDM (B). Small IBDM is decreased in $Mdr2^{-/-}$ mice following treatment with the H1HR antagonist when compared to saline treated mice. Large IBDM is decreased in $Mdr2^{-/-}$ mice following treatment with the H2HR antagonist when compared to saline treated mice. $Mdr2^{-/-}$ mice treated with both the H1HR and H2HR antagonists do not have a change in small IBDM, but have a significant reduction in large IBDM when

compared to saline treated mice (B). Data are mean ± SEM of 12 cumulative experiments from 6-8 mice per group. *p<0.05 versus WT; #p<0.05 versus small ducts in $Mdr2^{-/-}$ + saline; &p<0.05 versus large ducts in $Mdr2^{-/-}$ + saline.

Supplemental Figure 3 Analysis of hepatic fibrosis and HSC activation in $Mdr2^{-/-}$ mice. Semi-quantification of Sirius Red/Fast Green staining was performed (A) and real-time PCR for fibronectin (B), collagen type-1a (C) and SYP-9 (marker of activated HSCs) (D) in total liver from $Mdr2^{-/-}$ treatment groups. Collagen deposition increased in $Mdr2^{-/-}$ mice treated with saline when compared to WT mice; however, treatment with the H2HR antagonist significantly decreased collagen deposition in $Mdr2^{-/-}$ mice when compared to saline treated mice. The degree of collagen deposition was unchanged in $Mdr2^{-/-}$ mice treated with the H1HR antagonist when compared to saline treated mice (A). The expression of fibronectin, collagen type-1a and SYP-9 is increased in total liver from $Mdr2^{-/-}$ mice treated with saline compared to WT; however, treatment with the H1HR antagonist, H2HR antagonist or both significantly reduced the expression of all of the aforementioned markers (D). Data are mean ± SEM of 12 cumulative experiments for Sirius Red/Fast Green and at least 6 experiments for real-time PCR. *p<0.05 versus WT; #p<0.05 versus $Mdr2^{-/-}$ saline treatment.

Supplemental Figure 4 *In vitro* evaluation of hHSC activation following treatment with either the H1HR or H2HR antagonist. Expression of the H1HR and H2HR were evaluated in cultured hHSCs and compared to cultured mast cells and cholangiocytes. hHSCs have increased expression of H1HR, but little to no expression of H2HR, when compared to

cultured mast cells and cholangiocytes, as demonstrated by RT-PCR (A) and qPCR (B). hHSCs were treated with either the H1HR or the H2HR antagonist (25 μM) for up to 72 hours before activation was evaluated. While hHSCs express both H1HR and H2HR, treatment with either antagonist did not affect activation as shown by qPCR for collagen-1a (C). Data are mean ± SEM of 6 experiments for qPCR. *p<0.05 versus basal.

Supplemental Figure 5 vWF and EMT evaluation by immunohistochemistry in human CCA. The expression of vWF, CK-7, E-Cadherin and vimentin are increased in tumors from human patients with CCA when compared to non-malignant tissues. Images are 20X magnification.

Supplemental Figure 6 *In vitro* evaluation of pooled mouse cholangiocyte proliferation following treatment with either the H1HR or H2HR antagonist. Pooled mouse cholangiocytes were treated with either the H1HR or H2HR antagonists (25 μM), *in vitro* for up to 48 hours and proliferation was measured by BrdU incorporation (BrdU-positive cells shown in red) (A) and MTS assay (B). Treatment with either the H1HR antagonist or H2HR antagonist decreased cholangiocyte proliferation compared to basal treatment. Data are mean ± SEM of 20 experiments for BrdU incorporation and 12 experiments for MTS assay. *p<0.05 versus basal. Representative images are 20X magnification.

Supplemental Figure 7 *In vitro* evaluation of proliferation, invasion and EMT in cultured human CCA cells. Proliferation, invasion and angiogenesis in cultured human CCA cells decreased following treatment with either H1HR or H2HR antagonists (25 μM)

for up to 48 hours when compared to basal treatment as shown by MTS assay (A), invasion assay (B) and real-time PCR for vWF (C). In contrast, E-Cadherin expression increased following treatment with H1HR or H2HR antagonists compared to basal treatment (D). Data are mean ± SEM of 6 experiments for MTS and invasion assay and 9 experiments for real-time PCR. *p<0.05 versus basal.

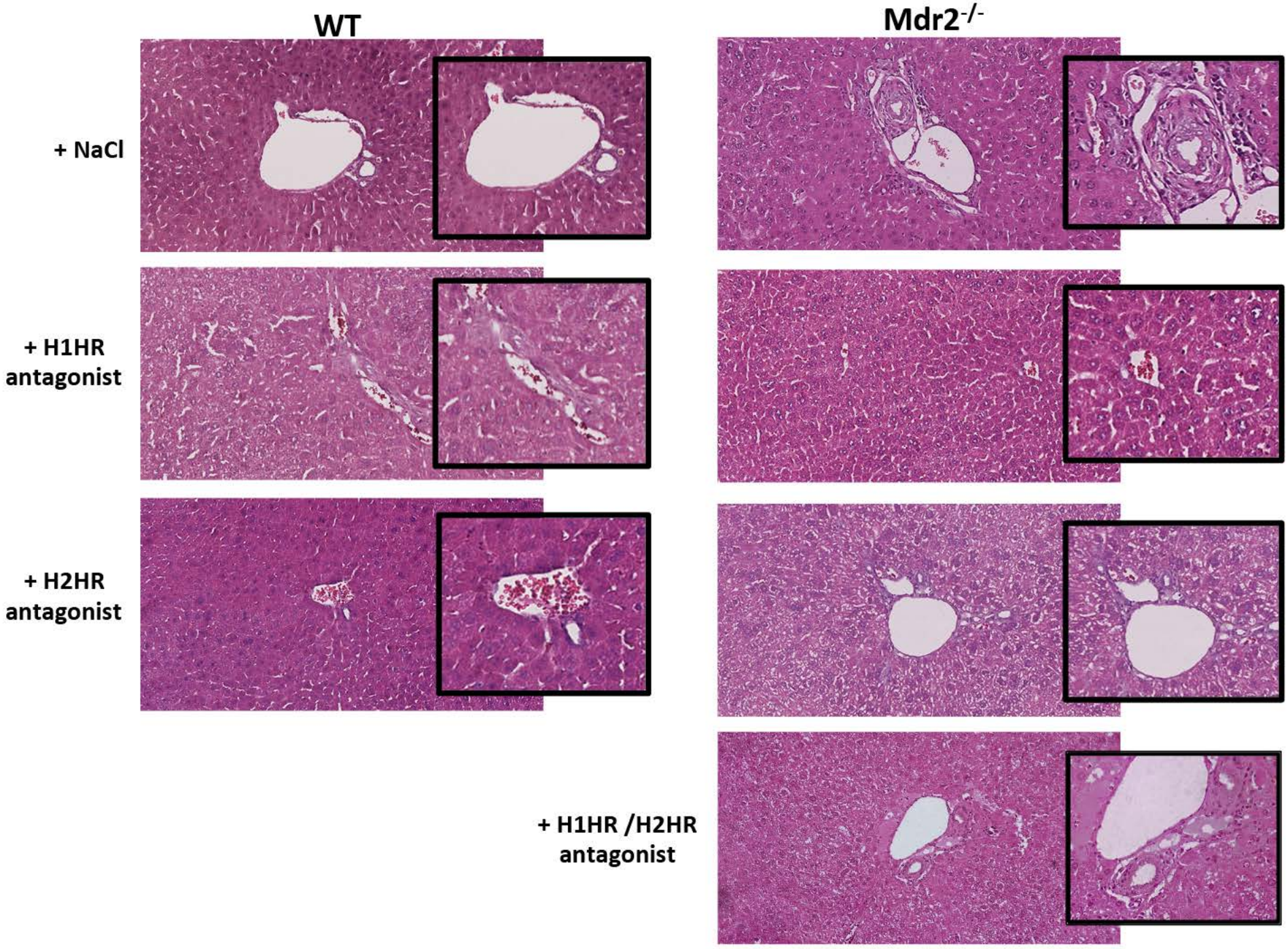
Supplemental Figure 8 Mast cell, cholangiocyte and CCA co-culture experiments. Mast cells were pretreated with either the H1HR or the H2HR antagonists (25 μM) for up to 72 hours and supernatants (conditioned medium) were collected. Cholangiocytes were treated with mast cell supernatants and proliferation was measured by BrdU incorporation (BrdU-positive cells shown in red) (A) and MTS assay (B). Pooled cholangiocytes treated with mast cell basal supernatants had increased proliferation when compared to basal (no mast cells). Co-culture with mast cells treated with either the H1HR or H2HR antagonist decreased biliary proliferation when compared to basal mast cell treatment (A, B). Similarly, CCA cells treated with mast cell basal supernatants had increased proliferative capacity compared to basal (no mast cells) and when CCA were stimulated with mast cell supernatants pre-treated with either H1HR or H2HR antagonists, CCA proliferation decreased (C). Data are mean ± SEM of 20 experiments for BrdU incorporation and 12 experiments for MTS assay. *p<0.05 versus basal; #p<0.05 versus mast cell basal supernatant treatment. Representative images are 20X magnification.

Supplemental Figure 9 *In vitro,* small and large cholangiocytes were evaluated for changes in IP_3 and cAMP levels following inhibition of H1HR or H2HR. Small and large

cultured cholangiocytes were treated with 0.1% BSA (basal), histamine (10 μM), mepyramine (25 μM) or histamine plus mepyramine for up to 48 hours and IP_3 levels were measured by EIA. Small cholangiocyte IP3 levels increase with histamine treatment that is blocked when pre-treated with the H1HR antagonist, mepyramine (A). Large cholangiocyte IP_3 levels were unchanged (data not shown). Small and large cholangiocytes were treated with 0.1% BSA (basal), forskolin (25 μM) or ranitidine (25 μM) for up to 48 hours prior to evaluating cAMP levels by EIA. Large (but not small) cholangiocyte cAMP levels increased following forskolin treatment and this was reduced in large cholangiocytes treated with the H2HR inhibitor, ranitidine (B). Data are mean ± SEM of 18 experiments for IP_3 and 24 experiments for cAMP assay. *$p<0.05$ versus basal; #$p<0.05$ versus histamine or forskolin treatment.

Supplemental Figure 10 Tumors from nu/nu mice treated with saline, H1HR or H2HR antagonists were evaluated for Notch/Jagged signaling by immunohistochemistry. Notch 1 and 2 expression was increased in tumors from nu/nu mice treated with saline; however, the expression of both Notch 1 and 2 decreased in tumors from mice treated with H1HR or H2HR antagonists (A). Similarly, Jagged 1 expression was increased in tumors from nu/nu mice treated with saline; however, the expression of Jagged 1 decreased in tumors from mice treated with H1HR or H2HR antagonists (B). Representative images are 20X magnification.

Supplemental Figure 1



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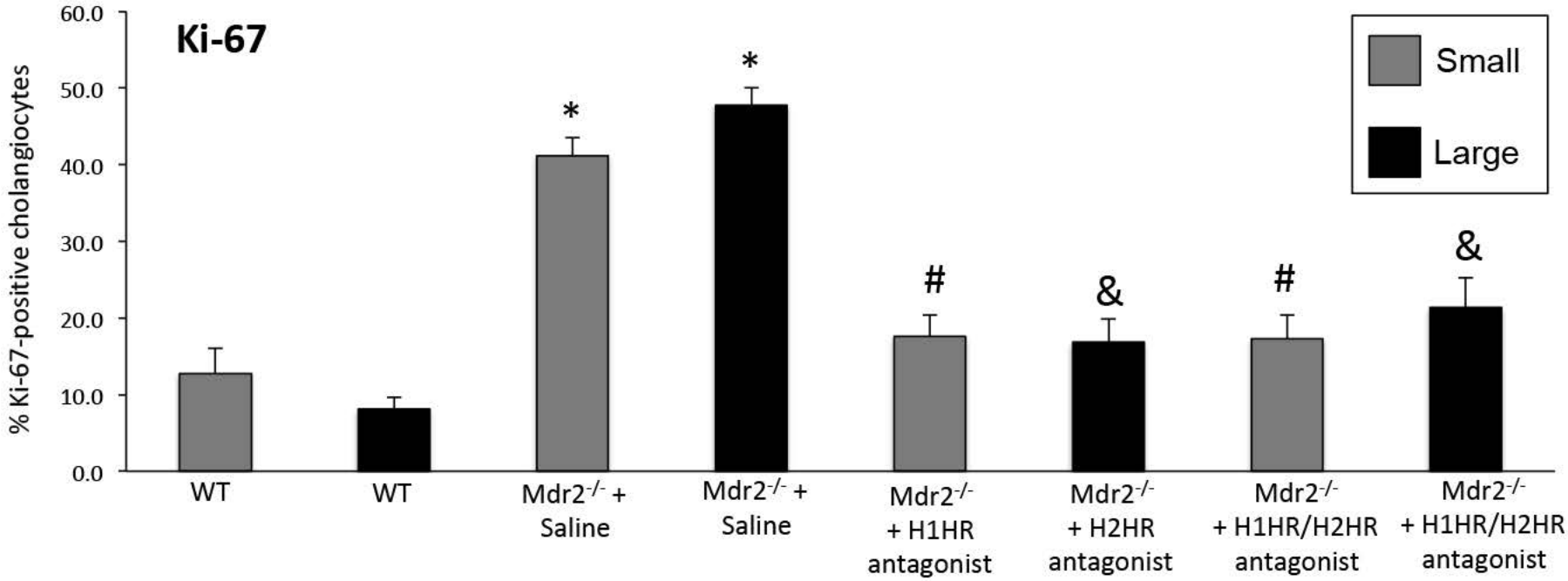

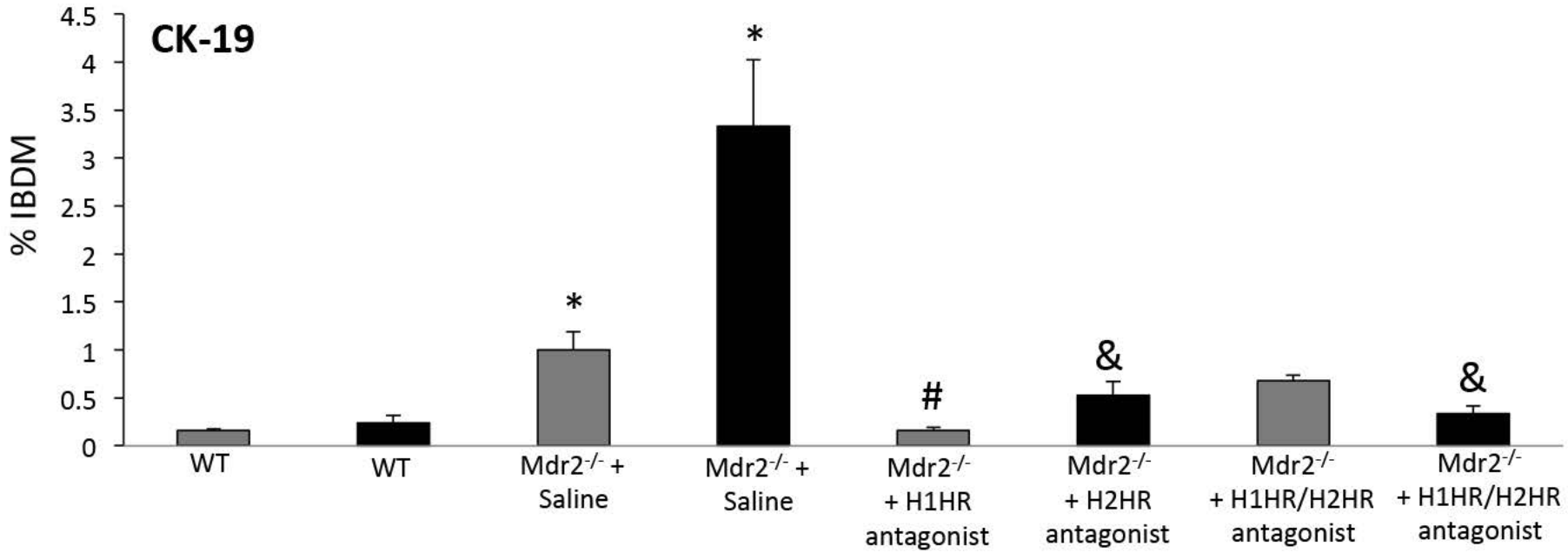

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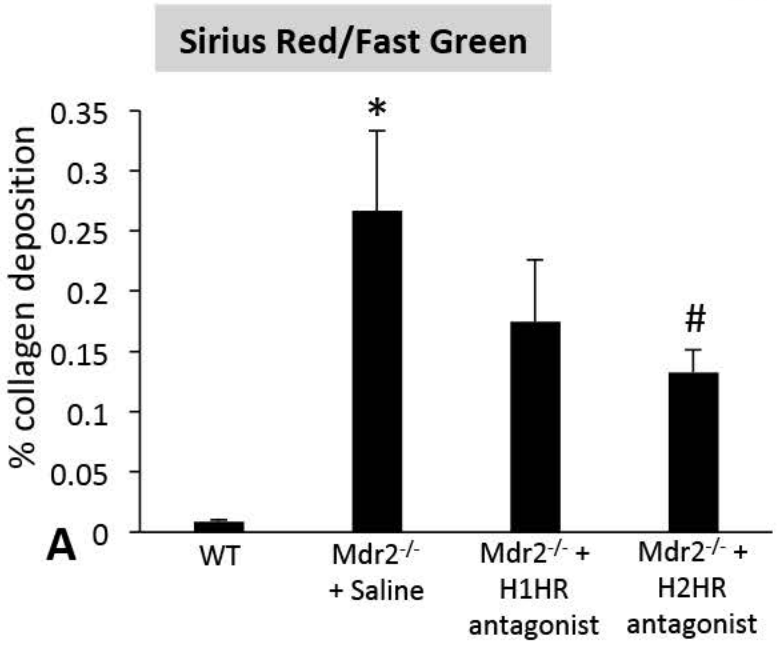

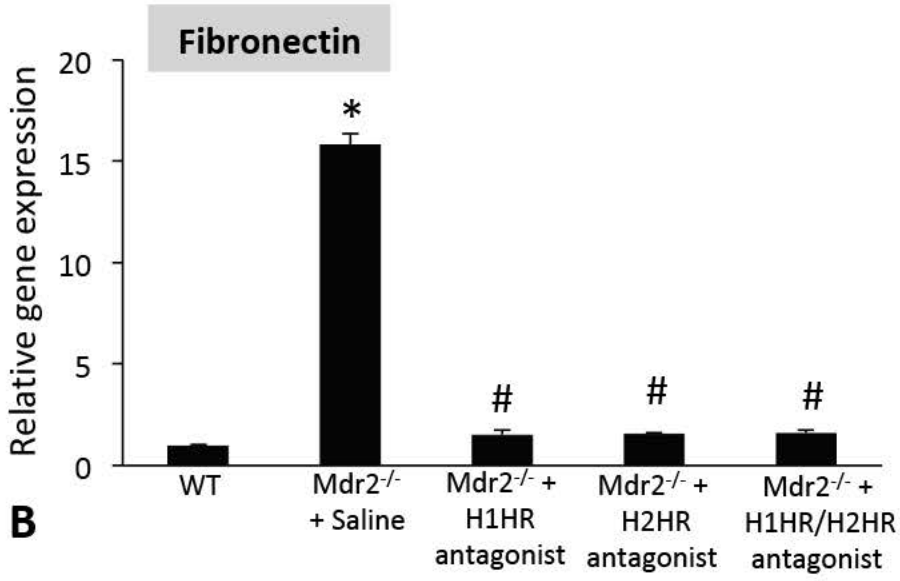

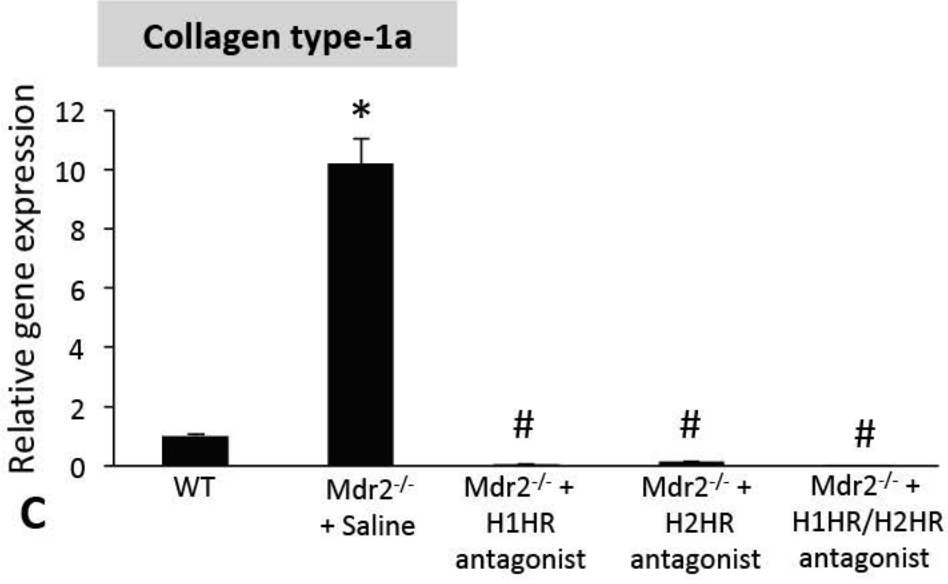

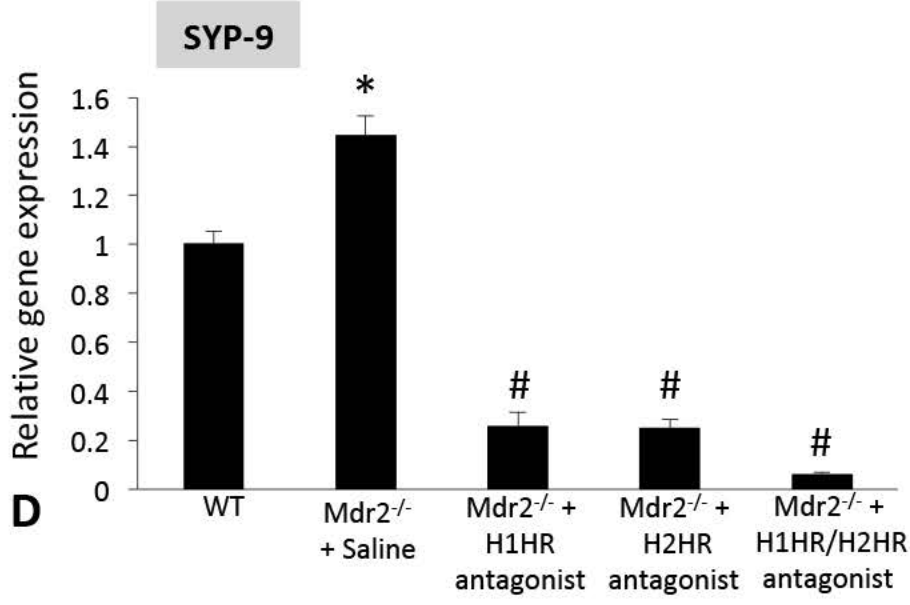

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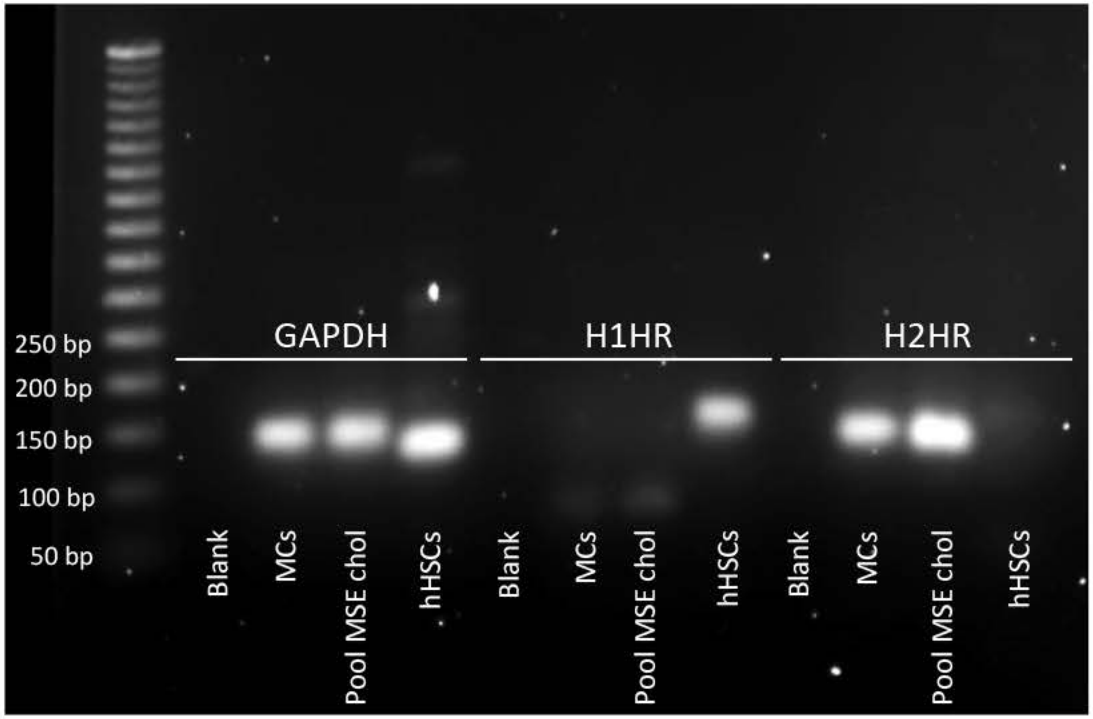

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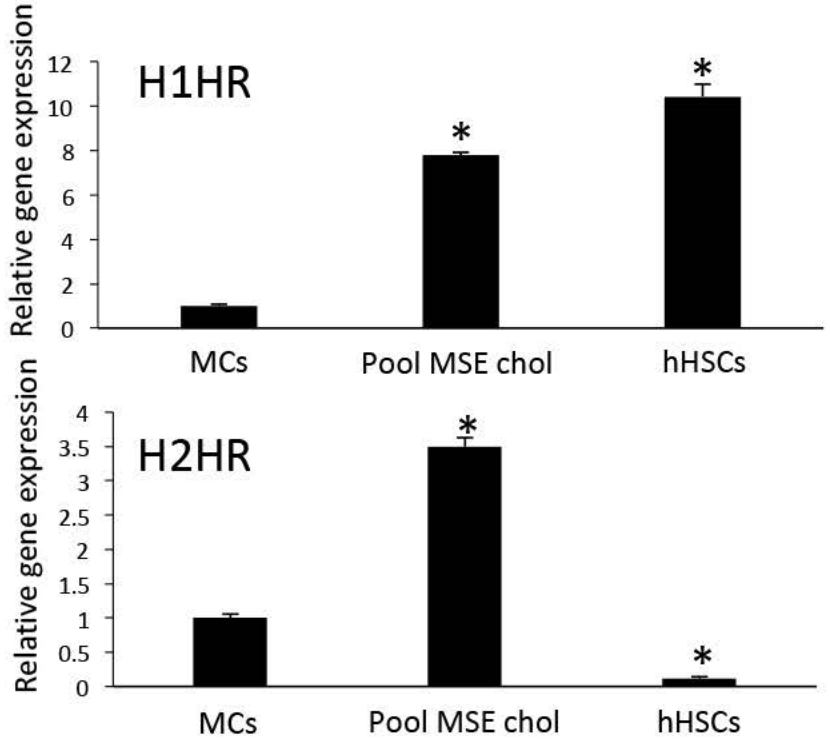

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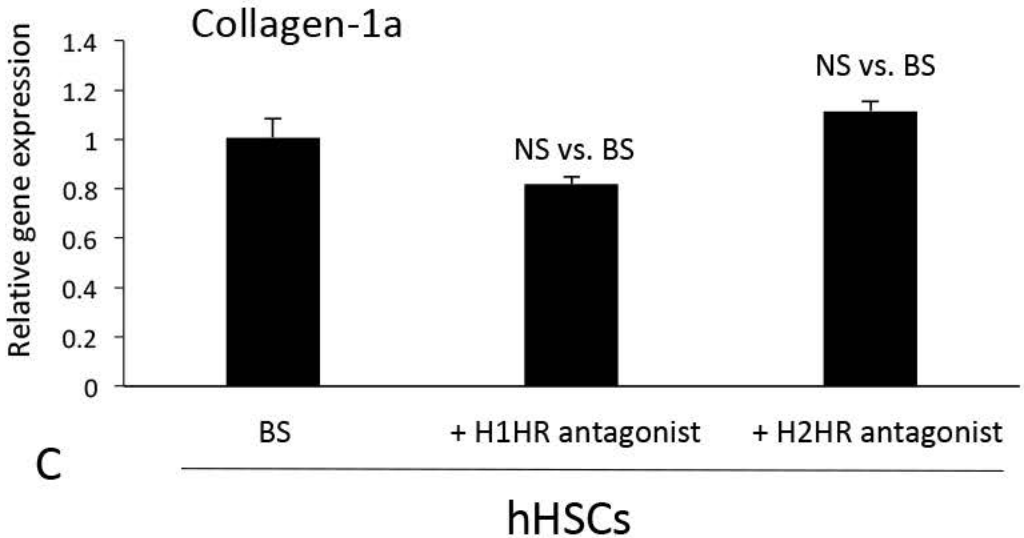

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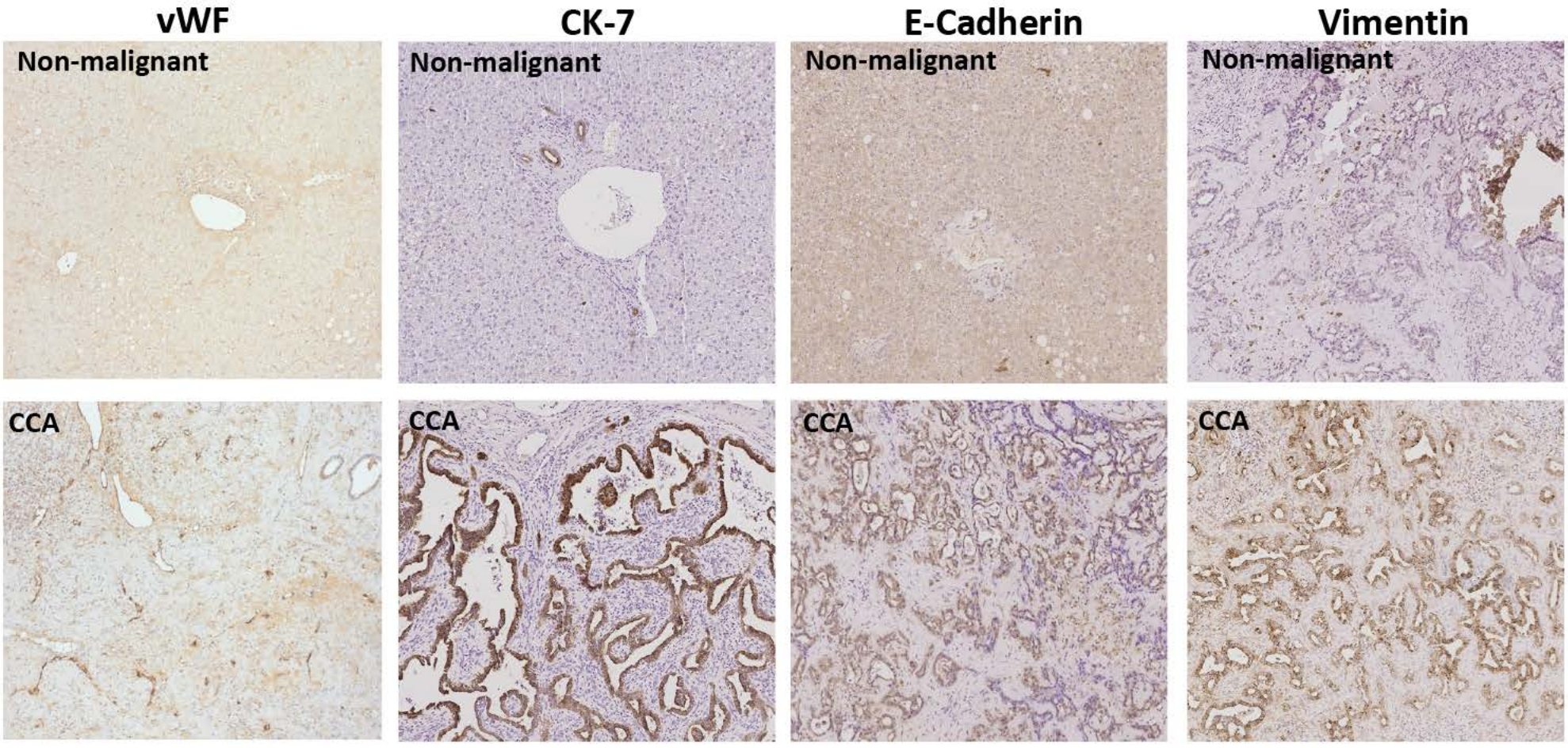
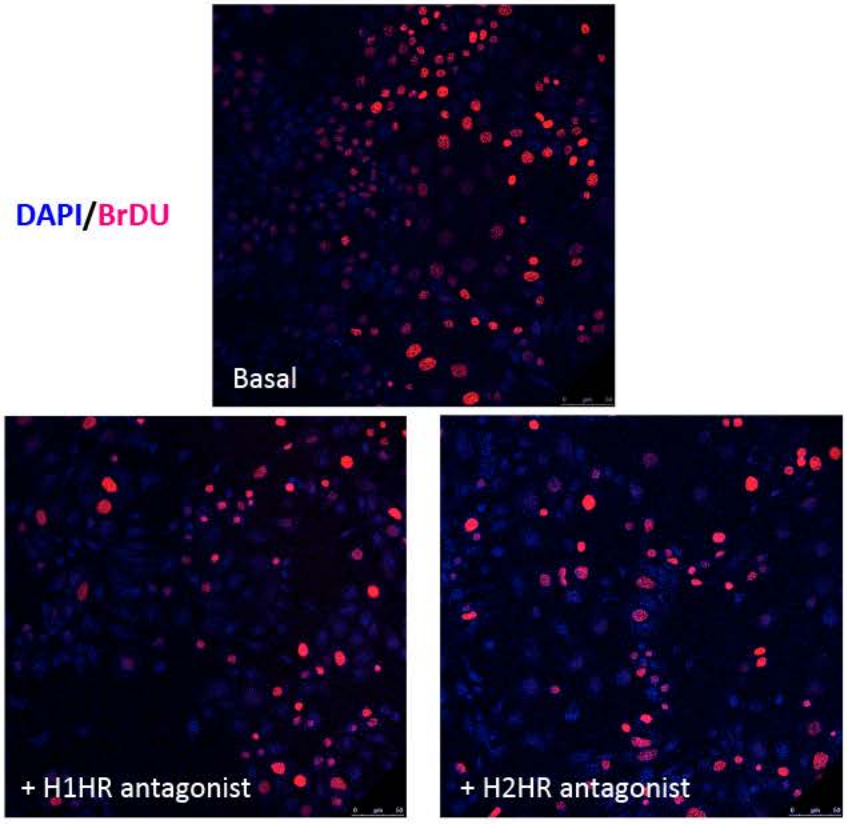
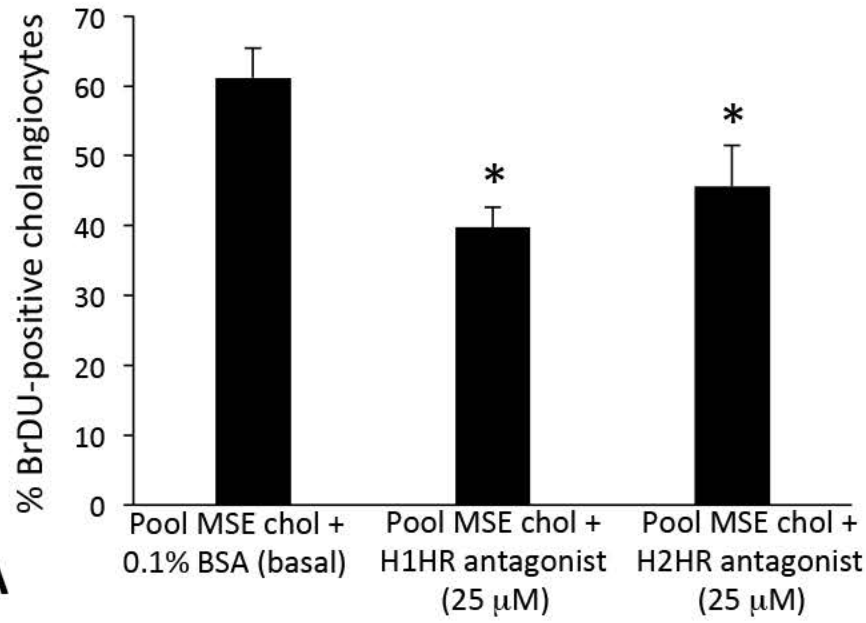
Supplemental Figure 5



Supplemental Figure 6





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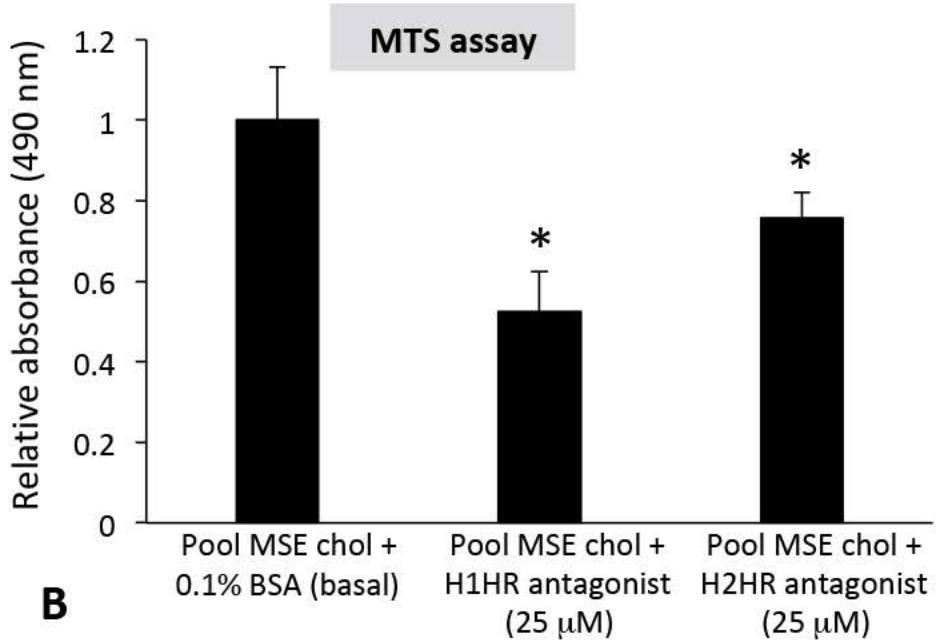

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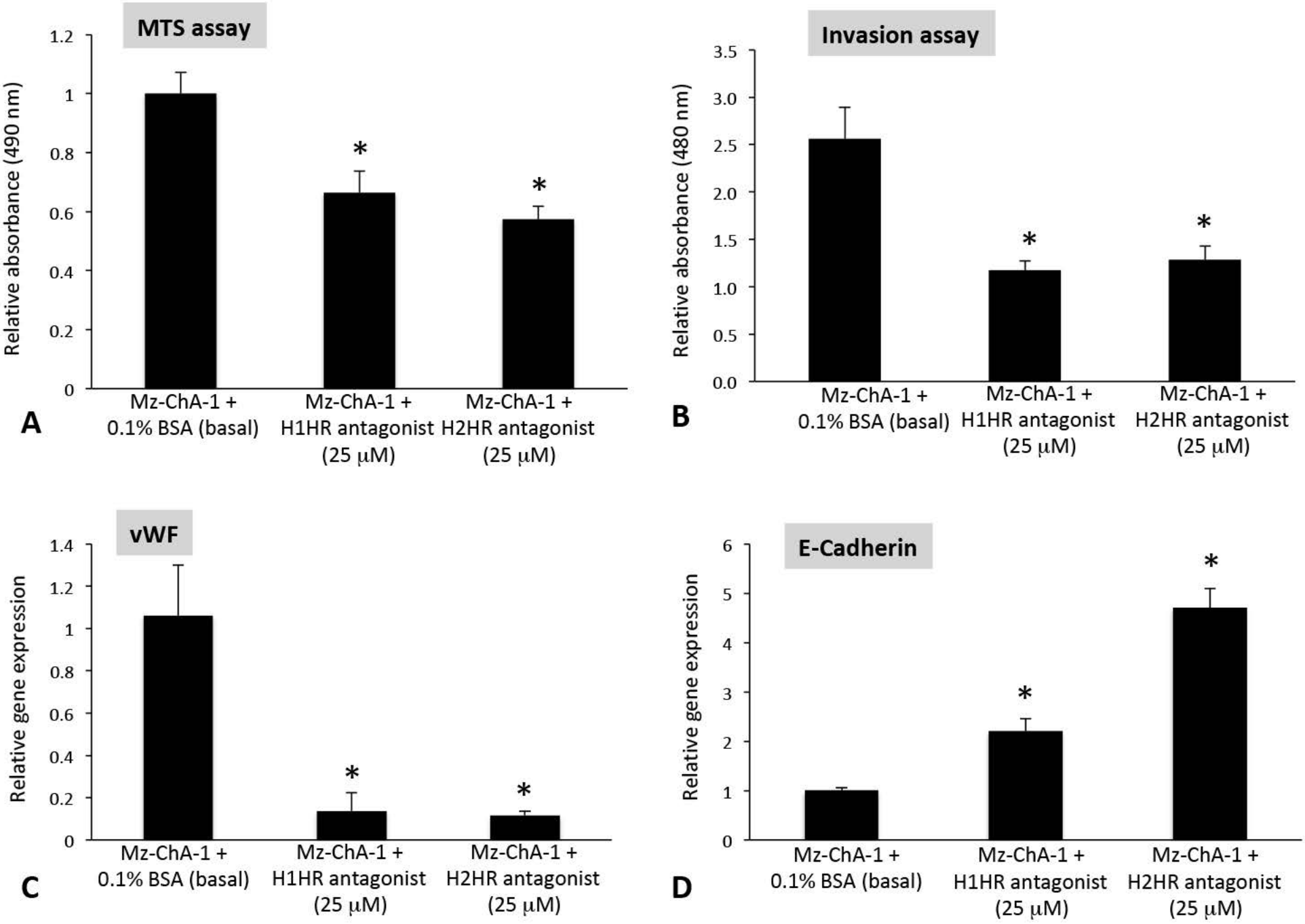
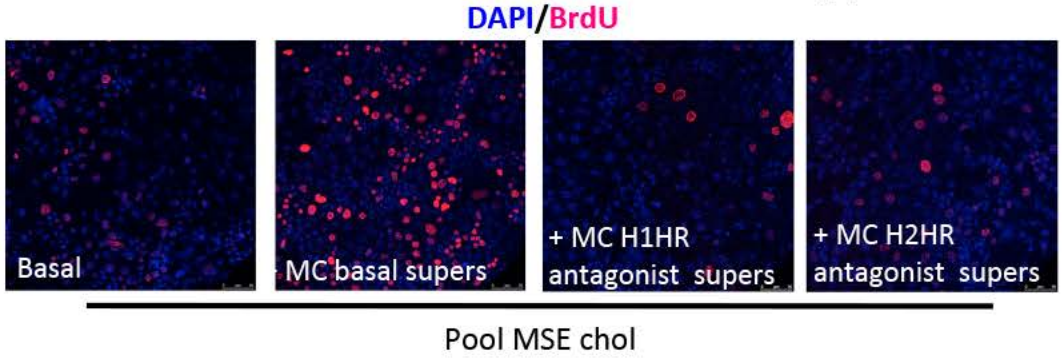
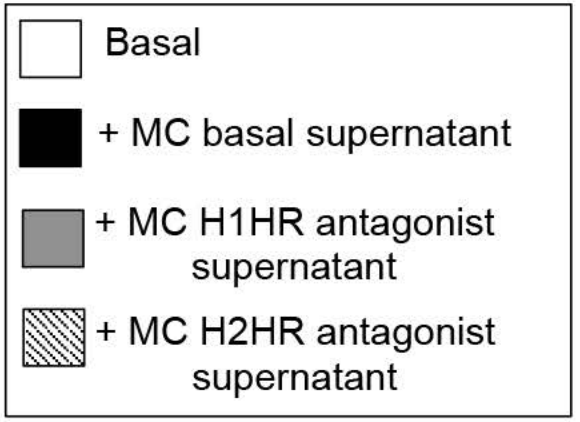
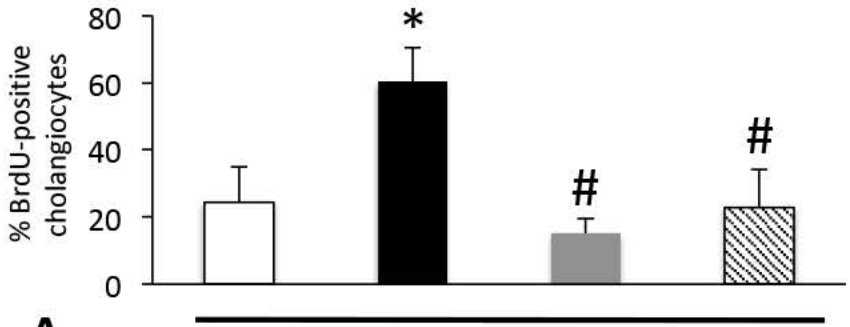


Supplemental Figure 8



Pool MSE chol





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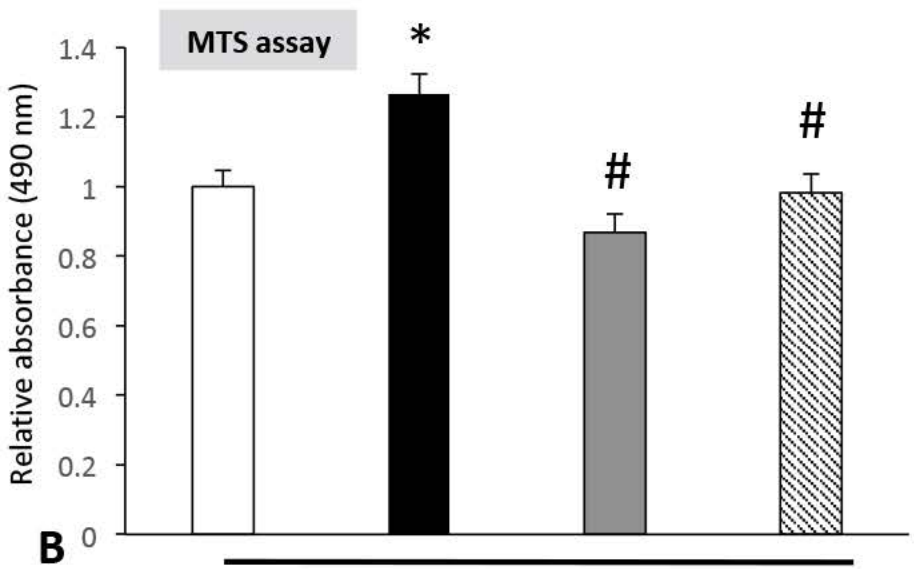

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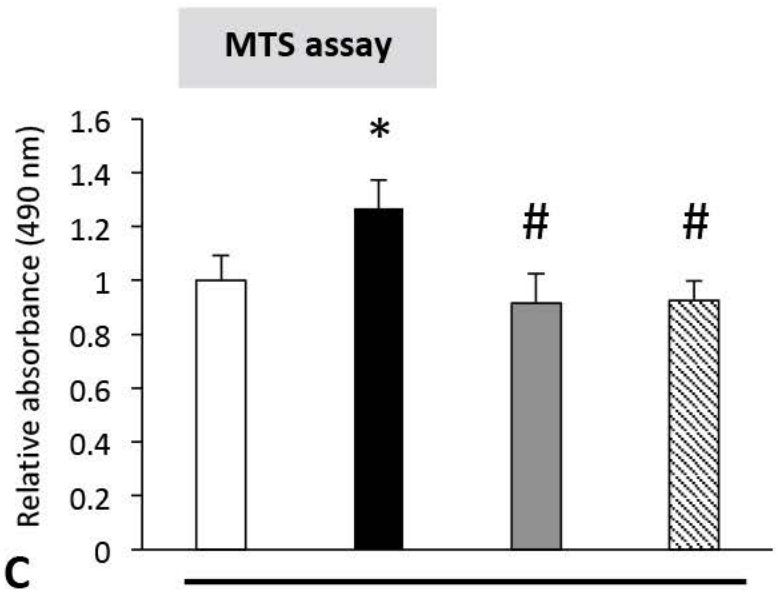
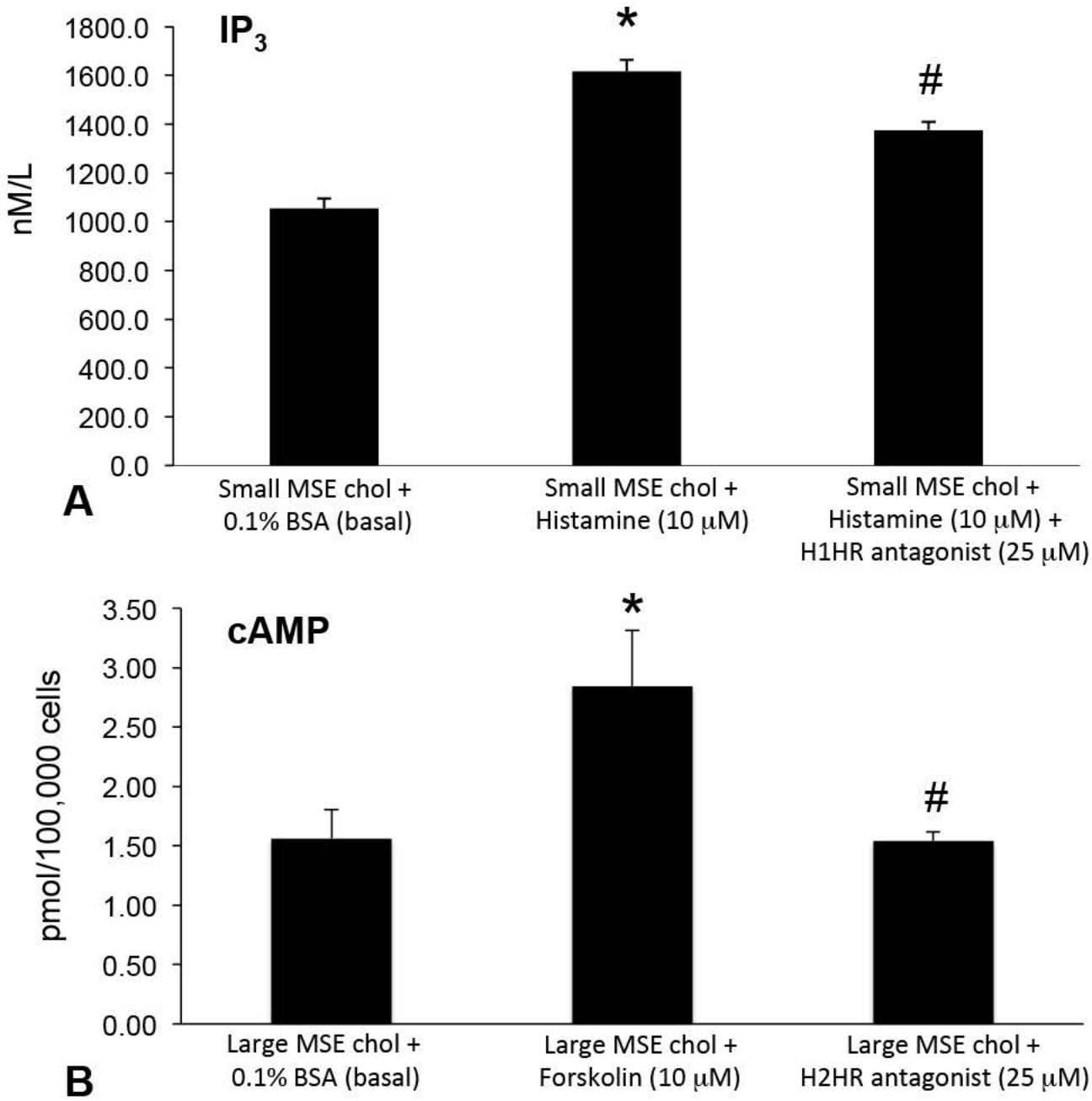


C

Mz-ChA-1

Supplemental Figure 9



Supplemental Figure 10