

## **SUPPLEMENTAL MATERIALS**

### **The Soluble Guanylate Cyclase Stimulator IWP-953 Increases Conventional Outflow Facility in Mouse Eyes**

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**Supplemental Tables: 2**

**Supplemental Table 1**

IWP-953 ( $\mu$ M)	Myofibroblast-like TM Cells		Endothelial-like TM Cells	
	HTM120 cGMP (pmol/mg protein)	HTM123 cGMP (pmol/mg protein)	HTM130 cGMP (pmol/mg protein)	HTM141 cGMP (pmol/mg protein)
0	9 $\pm$ 2	28 $\pm$ 0	17 $\pm$ 3	3 $\pm$ 0
0.3	16 $\pm$ 2	91 $\pm$ 36	24 $\pm$ 5	17 $\pm$ 3
1	28 $\pm$ 5	112 $\pm$ 11	25 $\pm$ 11	40 $\pm$ 3
3	36 $\pm$ 4	83 $\pm$ 27	23 $\pm$ 4	22 $\pm$ 11
10	56 $\pm$ 6	79 $\pm$ 42	15 $\pm$ 9	32 $\pm$ 4

Cyclic GMP production in primary cultures of human trabecular meshwork (HTM) cells stimulated by IWP-953. Myofibroblast-like HTM120 and HTM123, and endothelial-like HTM130 and HTM141 cell cultures were seeded at a density of  $1 \times 10^5$  cells/well in 24-well plates and incubated with DMEM containing IBMX (0.5 mM) for 15 minutes at 37°C. HTM cell cultures were then incubated with increasing concentrations of IWP-953 ranging from 0.3  $\mu$ M to 10  $\mu$ M or vehicle (0.5% DMSO) for 15 minutes at 37°C. Following cell lysis, cleared HTM cell supernatants were acetylated and the amount of cGMP was measured using a cGMP enzyme immunoassay. Data are expressed as the mean  $\pm$  SD ( $N = 3$ ).

**Supplemental Table 2**

IWP-953 ( $\mu$ M)	Myofibroblast-like TM Cells		Endothelial-like TM Cells	
	HTM120 cGMP (nM)	HTM123 cGMP (nM)	HTM130 cGMP (nM)	HTM141 cGMP (nM)
0	1	1	1	3
0.3	1	3	4	8
1	2	8	5	14
3	2	8	5	15
10	2	7	8	17

Cyclic GMP secretion by primary cultures of human trabecular meshwork (HTM) cells, following treatment with IWP-953. Myofibroblast-like HTM120 and HTM123, and endothelial-like HTM130 and HTM141 cell cultures were treated with increasing concentrations of IWP-953 (0.3  $\mu$ M to 10  $\mu$ M) or vehicle (0.5% DMSO). HTM cell culture supernatants were acetylated and cGMP content was measured using a cGMP enzyme immunoassay. Data are expressed as the mean ( $N = 3$ ). Due to the small amount of secreted cGMP following treatment with IWP-953, no SD is shown.