

Supplemental Figures and Tables

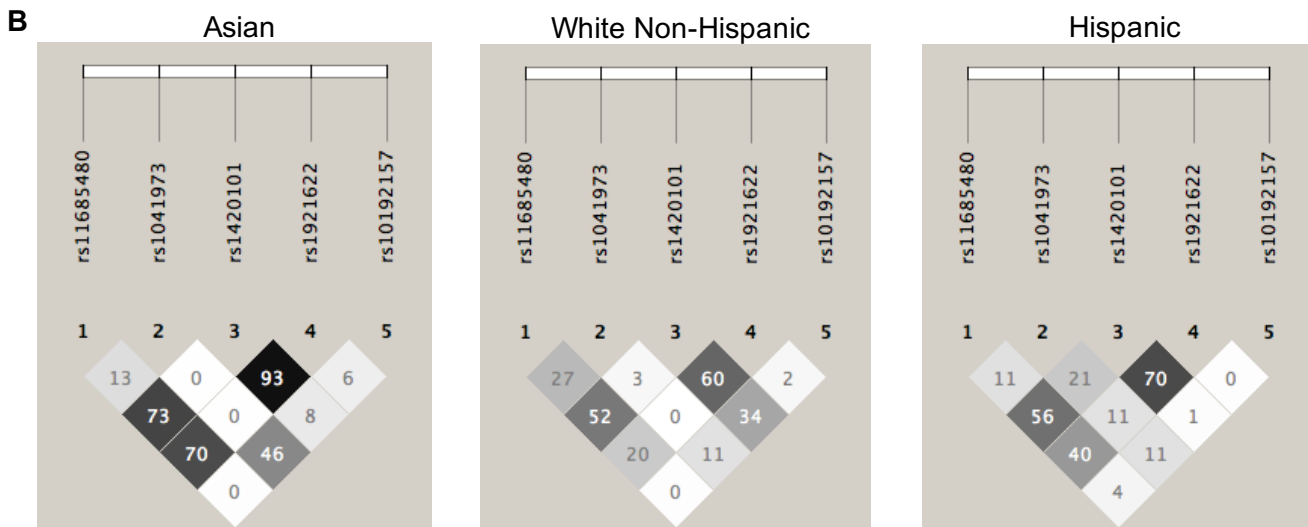
IL1RL1 Asthma Risk Variants Regulate Airway Type 2 Inflammation

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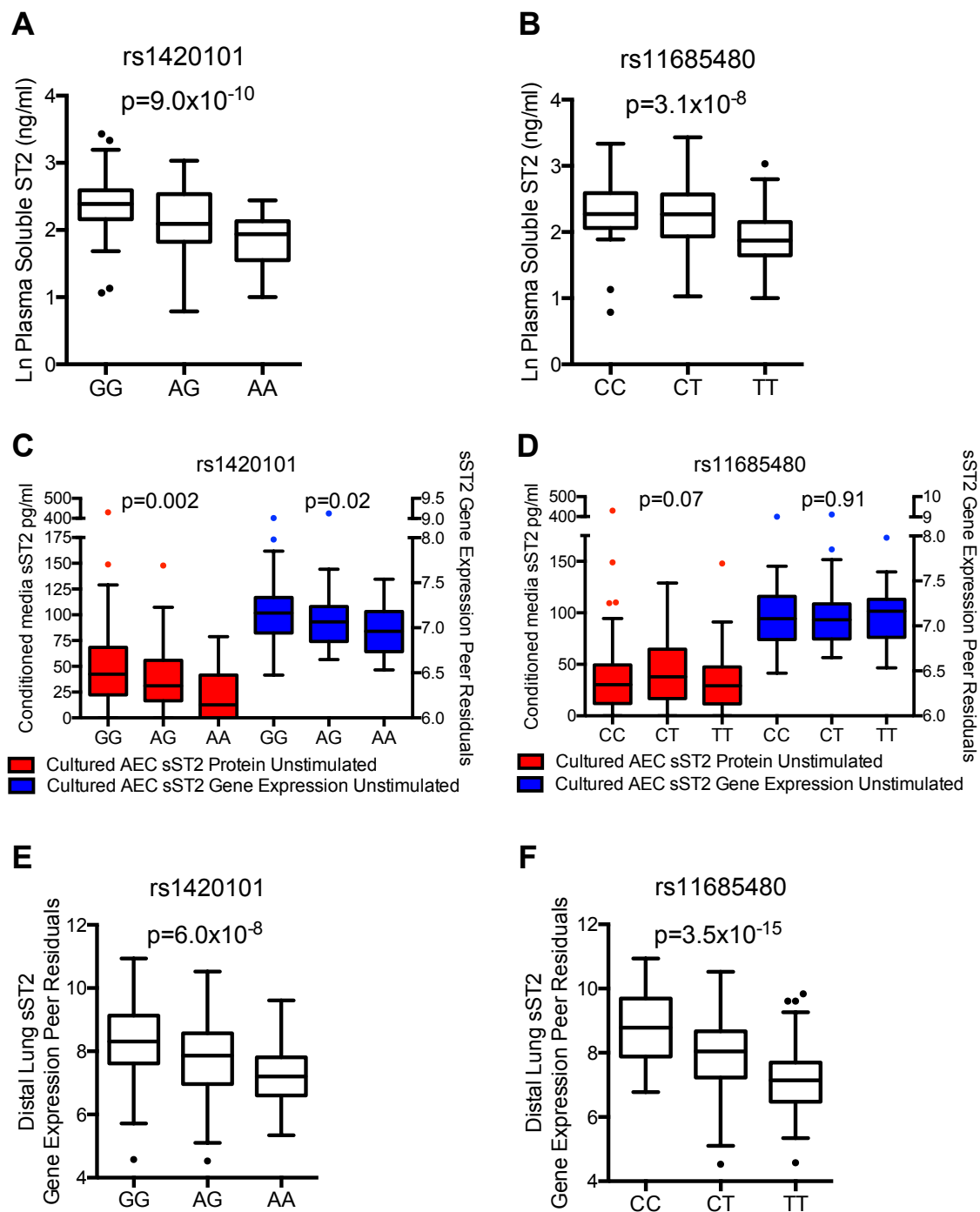
<i>IL1RL1</i> single nucleotide polymorphisms (SNPs) tested and prior disease or functional implication					
Variant	Minor Allele Frequency in UCSF Plasma Cohort				Location
	White Non-Hispanic (N=90)	Asian (N=32)	Hispanic (N=28)	Black (N=13)	
rs11685480 (T)	0.57	0.53	0.32	0.35	Distal Promoter
rs1041973 (T)	0.28	0.13	0.20	0.38	Exon 3
rs1420101 (A)	0.43	0.45	0.27	0.25	Intron 5
rs1921622 (T)	0.57	0.48	0.28	0.23	Intron 10
rs10192157 (A)	0.32	0.09	0.29	0.58	Exon 11

Disease or Functional Implication:
 rs11685480 (T): Associated with atopic dermatitis in Japanese individuals and 3-SNP distal promoter haplotype altered expression in vitro (Shimizu et al, Human Mol Gen, 2005); Associated with serum sST2 in pediatric Dutch cohort (Savenije et al, JACI, 2011)
 rs1041973 (T): Associated with asthma in Mexican population (Wu et al, JACI, 2010); Associated with asthma, eosinophils and serum ST2 in pediatric Dutch cohort (Savenije et al, JACI, 2011)
 rs1420101 (A): GWAS of peripheral blood eosinophils, asthma, atopic asthma in European and East Asian populations (Gudbjartsson et al, Nat Gen, 2009); Associated with serum sST2 in pediatric Dutch cohort (Savenije et al, JACI, 2011); GWAS of serum sST2 (Ho et al, JCI, 2013)
 rs1921622 (T): Associated with asthma, serum IgE, and bronchial hyperreactivity in Dutch asthma families (Reijmerink et al, JACI, 2008); Associated with serum sST2 in pediatric Dutch cohort (Savenije et al, JACI, 2011); Associated with severe RSV infection in Dutch children age less than 1 year old (Faber et al, PLOSOne, 2012)
 rs10192157 (A): Coding haplotype increased signaling through ST2L in KU812 cell line (Ho et al, JCI, 2013); Coding haplotype protective for asthma and reduced signaling through ST2L in HEK cell line (Ramirez-Carrozzi et al, JACI, 2014)



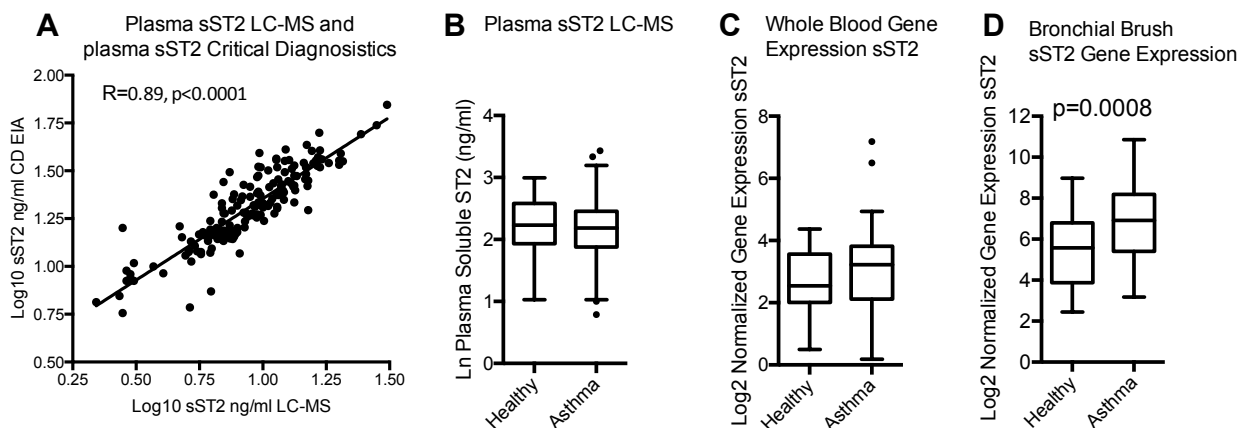
Supplemental Figure 1. Candidate *IL1RL1* Single Nucleotide Polymorphisms (SNPs) have been implicated in the pathogenesis of asthma and allergic disease. (A) Table showing the 5 candidate SNPs tested in our UCSF cohort, the minor allele frequencies in the UCSF plasma cohort stratified by racial group, and prior functional and disease implications. (B) Linkage disequilibrium

maps for the 5 candidate SNPs in our UCSF plasma cohort stratified by racial group (data not shown for blacks due to limited numbers of subjects in that group).



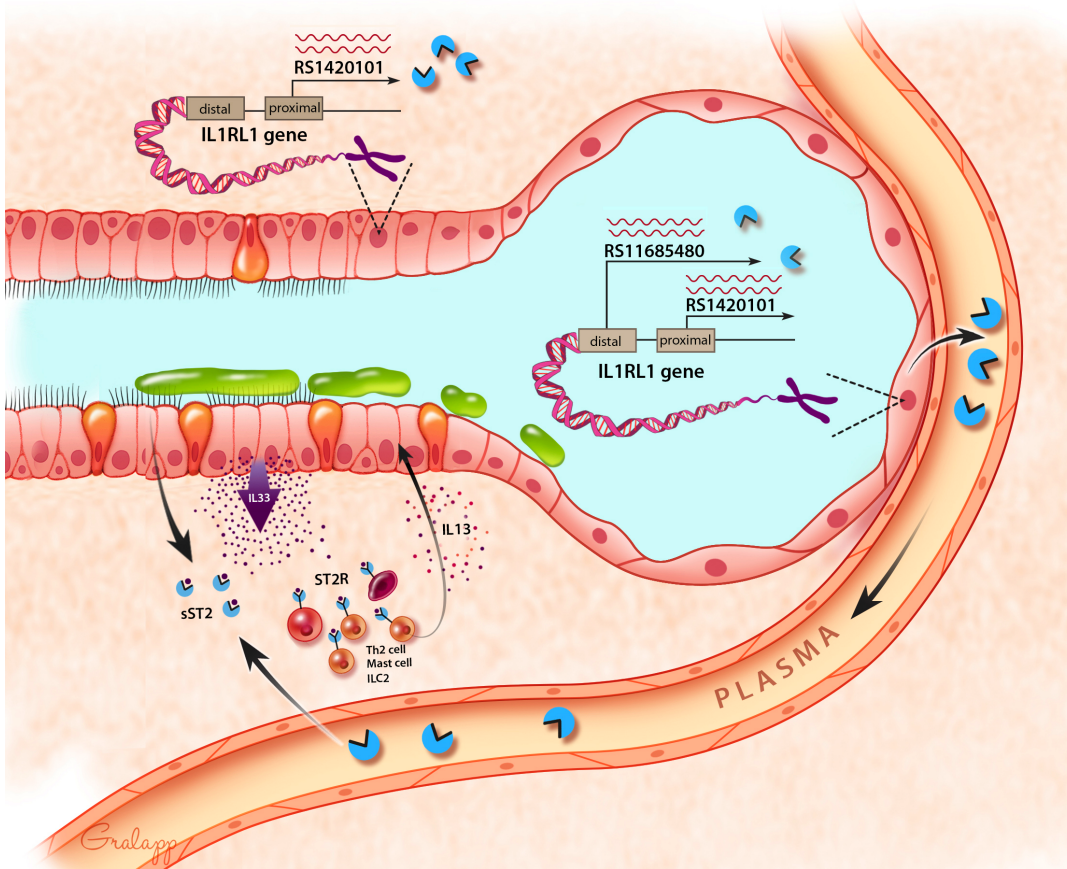
Supplemental Figure 2. rs1420101 and rs11685480 risk alleles are associated with lower sST2 plasma protein levels and lung sST2 expression. (A, B) Asthma risk allele A for rs1420101 (N=164) and T for 11685480 (N=171) are associated with lower sST2 plasma protein levels. **(C, D)** Risk allele A for rs1420101, but not 11685480 genotype, is associated with lower sST2 protein

measures (N=141, red) and sST2 gene expression (N=127, blue) in cultured human primary airway epithelial cells (AEC) in the unstimulated condition. **(E, F)** Risk allele A for rs1420101 and T for 11685480 are associated with lower sST2 gene expression (N=237) distal lung parenchymal tissue from the Genotype-Tissue Expression Project (GTEx) dataset. AEC and GTEx gene expression data is shown as peer residuals generated as described in the methods. Data presented as Tukey box plots with box containing the first and third quartile, line at median, and whiskers at 1.5 interquartile range. All p values generated from additive linear regression models.



Supplemental Figure 3. sST2 protein and sST2 gene expression in the blood do not differ between healthy and asthmatic subjects while sST2 gene expression in the airway does. (A) Liquid chromatography-mass spectroscopy (LC-MS) measures of sST2 are tightly correlated to measures of sST2 by Critical Diagnostics Enzyme Immunoassay (CD EIA). N=171, p value reflects simple linear regression. **(B)** Plasma sST2 levels as measured by LC-MS do not differ between asthmatics (N=124) and healthy controls (N=47). **(C)** Gene expression of sST2 in whole blood cell RNA is not different in asthmatics (N=63) and healthy controls (N=23). **(D)** Gene expression of sST2 in bronchial brush RNA is increased in asthmatics (N= 83) compared to healthy controls (N=22). Data

presented as Tukey box plots with box containing the first and third quartile, line at median, and whiskers at 1.5 interquartile range; comparisons performed by two-tailed t-test.



Supplemental Figure 4. Schematic depicting the molecular mechanism by which *IL1RL1* genetic variants drive type 2 inflammation in asthma. Expression quantitative trait loci (eQTL) in the proximal and distal promoters independently drive sST2 expression in the lung. rs1420101 or a tag of this SNP alters expression of sST2 produced by the proximal promoter in airway and alveolar epithelial cells. Alternatively, rs11685480 or a tag of this SNP alters sST2 expression through the distal promoter which is active in alveolar epithelial cells. sST2 produced by airway epithelial cells acts locally in the airway to inhibit IL-33 signaling and suppress type 2 inflammation. sST2 produced

in the distal lung by alveolar epithelial cells and microvascular endothelial cells accumulates in the plasma and acts as a reservoir, which leaks into injured tissues and again acts to suppress type 2 inflammation. A genetically determined deficiency in soluble ST2 both in the airway and in the plasma reservoir increases the risk of type 2 airway inflammation in asthma.

Supplementary Table S1. Characteristics of UCSF Clinical Cohort

Variable		Healthy (N=61) N (%)	Asthma (N=218) N (%)	P value
Plasma sST2 LCMS Measurement		47 (77.1)	124 (56.9)	
Epithelial Brush RNA sST2 Gene Expression		22 (36.1)	83 (38.1)	
Whole Blood RNA sST2 Gene Expression		23 (37.7)	63 (28.9)	
Type 2 Endotyping (Sputum and Epithelial Brush)		- -	150 (68.8)	
Sex				0.14
	Male	30 (49.2)	84 (38.5)	
	Female	31 (50.8)	134 (61.5)	
Race				
	American Indian/Alaska Native	1 (1.6)	6 (2.8)	
	Asian	13 (21.3)	27 (12.4)	
	Black or African American	4 (6.6)	23 (10.6)	
	White	38 (62.3)	137 (62.8)	
	NHOPI	0 (0)	3 (1.4)	
	Mixed	4 (6.6)	17 (7.8)	
	Unknown/decline	1 (1.6)	5 (2.3)	
Ethnicity				
	Non-Hispanic	55 (90.2)	173 (80.5)	
	Hispanic	6 (9.8)	42 (19.5)	
Currently on an inhaled corticosteroid				
	Yes	- -	112 (51.6)	
	No	- -	105 (48.4)	
Age, median (IQR)		32 (27-44)	33 (25-47)	0.6
FEV-1 % predicted, median (IQR)		95.5 (91.3-104.7)	83.6 (72-92.3)	< 0.0001
Blood eosinophils (x106/L), median (IQR)		120 (70-180)	260 (150-420)	< 0.0001
IgE, median (IQR)		26 (13-49)	213 (75.5-516)	< 0.0001
Average FeNO (ppb), median (IQR)		14.7 (10-19.4)	35 (18.8-62)	< 0.0001
PC20 (mg/ml), median (IQR)			0.6 (0.2-1.7)	

University of California, San Francisco (UCSF), soluble ST2 (sST2), Liquid Chromatography Mass Spectroscopy (LCMS), Native Hawaiian and Other Pacific Islander (NHOPI), Interquartile Range (IQR), Forced Expiratory Volume 1 second (FEV-1), Fraction of Exhaled Nitric Oxide (FeNO), parts per billion (ppb)

Supplementary Table 2. Characteristics of Type 2 Low vs High Asthmatics

Variable		Type 2 Low Asthma (N=63) N (%)	Type 2 High Asthma (N=87) N (%)	P value
Epithelial Brush Type 2 Endotyping		32 (50.8)	41 (47.1)	0.74
Sputum Cell Type 2 Endotyping		31 (49.2)	46 (52.9)	
Sex				0.4
	Male	23 (36.5)	38 (43.7)	
	Female	40 (63.5)	49 (56.3)	
Race				
	American Indian/Alaska Native	2 (3.2)	3 (3.5)	
	Asian	8 (12.7)	13 (14.9)	
	Black or African American	3 (4.8)	13 (14.9)	
	White	43 (68.3)	46 (52.9)	
	NHOPI	1 (1.6)	1 (1.2)	
	Mixed	5 (7.9)	9 (10.3)	
	Unknown/decline	1 (1.6)	2 (2.3)	
Ethnicity				
	Non-Hispanic	52 (82.5)	68 (80)	
	Hispanic	11 (17.5)	17 (20)	
Currently on an inhaled corticosteroid				0.74
	Yes	31 (49.2)	40 (46)	
	No	32 (50.8)	47 (54)	
Age, median (IQR)		31 (24-45)	33 (25-49)	0.47
FEV-1 % predicted, median (IQR)		86 (77.1-98.5)	81 (67-90)	0.018
Blood eosinophils (x106/L), median (IQR)		160 (110-330)	350 (250-500)	< 0.0001
IgE, median (IQR)		131 (57-313)	262 (96-547)	0.0061
Average FeNO (ppb), median (IQR)		23 (14.4-33.5)	56.5 (28.3-80.5)	< 0.0001
PC20 (mg/ml), median (IQR)		1 (0.3-3.3)	0.4 (0.2-0.9)	0.0007

Native Hawaiian and Other Pacific Islander (NHOPI), Forced Expiratory Volume 1 second (FEV-1), Interquartile Range (IQR), Fraction of Exhaled Nitric Oxide (FeNO), parts per billion (ppb)

Supplementary Table 3. Bronchial Brush sST2 Gene Expression Association with *IL1RL1* SNPs

All Races					
SNP Tested	Regression Coefficient	Confidence Intervals		P value	N
rs1420101	0.02	-0.08	0.12	0.71	103
rs11685480	0.02	-0.08	0.11	0.75	105
White Non-Hispanic					
SNP Tested	Regression Coefficient	Confidence Intervals		P value	N
rs1420101	0.01	-0.14	0.16	0.88	48
rs11685480	0.02	-0.13	0.18	0.77	48

Supplementary Table 4. Taqman Primer and Probe Sequences

Preamplification Primers			Taqman Primers and Probes	
sST2	FP RP	5-ATAGCACTGGTCACATTGCCTTAC-3 5-CAACAAATATTTAGCTGAGCACTGG-3	FP Probe RP	5-CTGCTCAACACGACTGCAAG-3 5-FAM-TCCATGAGGGCAGGGACATCATCTC-BHQ-3 5-GGCTAGCTGCCAGGTATTGC-3
PPIA	FP RP	5-ATGAGAACTTCATCCTAAAGCATAAG-3 5-TTGGCAGTGCAGATGAAAACT-3	FP Probe RP	5-ACGGGTCCTGGCATCTTGT-3 5-FAM-ATGGCAAATGCTGGACCCAACACA-BHQ-3 5-GCAGATGAAAACTGGGAACCA-3
RPL13A	FP RP	5-GGACCGTGCGAGGTATGCT-3 5-TTCAGACGCACGACCTTGAG-3	FP Probe RP	5-TATGCTGCCCCACAAAACC-3 5-FAM-CAGAGCGGCCTGGCCTCGCT-BHQ-3 5-TGCCGTCAAACACCTTGAGA-3
EEF1A1	FP RP	5-TGCTAACATGCCTTGTTCAAG-3 5-TTGGACGAGTTGGTGGTAGGAT-3	FP Probe RP	5-CCTTGTTCAAGGGATGGAA-3 5-FAM-CACTGGCATTGCCATCCTTACGGG-BHQ-3 5-GCCTCAAGCAGCGTGGTT-3
GAPDH	FP RP	5-CAATGACCCCTTCATTGACCTC-3 5-CTCGCTCCTGGAAGATGGTGAT-3	FP Probe RP	5-GATTCCACCCATGGCAAATTC-3 5-FAM-CGTTCTCAGCCTTGACGGTGCCA-BHQ-3 5-GGGATTTCATTGATGACAAGC-3
RPL37A	FP RP	5-TATGTGAAATAATCCGGACATGCT-3 5-TGCAATTGTAAGGCGTCTCTCT-3	FP Probe RP	5-CGGACATGCTTCTGCTATGG-3 5-FAM-CCCAGATTCGAAACTATGGCGACA-BHQ-3 5-GGCGTCTCTCTCATCCAGGT-3
Exon 1a			FP Probe RP	5-GAGAGGCTGGCTGTTGTATT-3 5-FAM-TCTGAGTTGTGAAACTGTGGGCAGA-BHQ-3 5-TGGGTTGTACTTGAGTTCTTTCT-3
Exon 1b			FP Probe RP	5-GGAGGGACCTACAAAGACTGG-3 5-FAM-AACTATTCTTAGCTCCGTCCTGA-BHQ-3 5-CAGAGGGGATGAACTTGAG-3