

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ferwerda B, Ferwerda G, Plantinga TS, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. *N Engl J Med* 2009;361:1760-7.

## Supplementary information to MS# NEJM-09-01053

### Supplementary information on methodologies

#### 1. Isolation of cDNA, DNA and development of primers.

RNA was isolated from whole blood using the PAXgene blood RNA kit (Qiagen) according to the protocol. Isolated RNA was thereafter treated with DNase (Ambion) and reverse transcribed to cDNA using oligo(dT) primers and MMLV reverse transcriptase (Invitrogen). PCR on the cDNA to investigate transcription of the different Dectin-1 isoforms was performed with primer pairs adapted from Meyer-Wentrup *et al* (2007).

DNA isolation was performed on whole blood using the isolation kit Puregene™ (Gentra system). DNA template of the Dectin-1 (also named CLEC-7A) sequence was taken from Genebank, chromosome position 12q13, NC\_000012.10 (10160643..10174135, complement). Primer3 software was used to develop all primers shown in Table 1. After amplification PCR products were cleaned using the kit High Pure PCR product purification kit (Roche) according to the manufacture protocol. Sequencing was performed at the Sequence Faculty at the department of human genetics Nijmegen.

Sequence for four out of six exons was obtained using both forward and reverse primers (Table S1). However, sequences for exon 3 and 4 were based on more than one forward sequences due to the lack of reverse primer specificity. No variations were detected in any exons other than exon 6.

**Supplementary Table 1**

Exon	Primer	Sequence (5' to 3')	[MgCl <sub>2</sub> ] (mM)	Annealing temperature
1	Dectin-1_205_FW	TTT-CAC-CAC-GTT-AGC-CAA-GCT	2.5	52 °C
	Dectin-1_205_RV	CTG-AAA-TAG-TTT-GCA-TCG-GTT		
2	Dectin-1_203_FW	CCC-TTT-ATA-AGT-GAA-ATG-GGC	1.75	60 °C
	Dectin-1_203_RV	ACC-GTG-CAA-GGC-CAG-ATT-TT		
3	Dectin-1_127_FW	GCC-AGT-GAT-AAA-TCA-GTT-ACT	3.5	56 °C
	Dectin-1_127_RV	TTC-TTC-TTC-TCC-ACC-TTC-TT		
3	Dectin-1_202_FW	TGG-CAA-CAT-TTT-CCC-TTC-TT	3.5	56 °C
	Dectin-1_202_RV	GCC-AAG-GGC-ATA-GTT-AAA-GG		
4	Dectin-1_301_FW	TCA-TTA-CCT-GGA-ATC-TCC-CTC-T	2.5	56 °C
	Dectin-1_301_RV	TGG-CAA-CTA-ATT-GGT-TAT-TTC-A		
5	Dectin-1_119_FW	GCT-GCT-CGA-CAG-AGG-TTT-TC	1.75	62 °C
	Dectin-1_119_RV	GGA-TGG-TCT-CGA-TCT-CCT-GA		
6	Dectin-1_106_FW	AAT-CAC-AGC-CTC-TCC-CTT-CA	2.5	60 °C
	Dectin-1_106_RV	GAT-TTA-AGC-CTC-CTT-TTC-CAA		

## **Legends to supplementary figures.**

**Supplementary Figure S1. Dectin-1 mutation leads to functional defects.** A. IL-6 production capacity of monocytes isolated from healthy volunteers (dectin-1 wt), patients heterozygous (dectin-1 het), or patients homozygous for the mutation (dectin-1 hom), and stimulated for 4h with heat-killed or live *C. albicans*. Each patient represents a white or a black bar. Variation in the control group is presented as SD (n=5). B. IL-6 production capacity of monocytes isolated from healthy volunteers (dectin-1 wt), patients heterozygous (dectin-1 het), or patients homozygous for the mutation (dectin-1 hom), and stimulated for 4h with the TLR4 antagonist LPS, or the TLR2 antagonist Pam3Cys. C. Synergism between dectin-1 and TLR2 stimulation of proinflammatory cytokines in cells isolated from control volunteers (dectin-1 wt) or patients homozygous (dectin-1 hom) for the stop codon mutation, after 24h stimulation at 37°C. (means  $\pm$  SD of three experiments, n=5 healthy volunteers, n=3 homozygous patients; \*p<0.05).

**Supplementary Figure S2. Dectin-1 deficiency does not impair *Candida* phagocytosis and killing.** Phagocytosis (A) and killing (B) of opsonized *C. albicans* by monocytes and neutrophils from healthy volunteers (five healthy volunteers without clinical signs of fungal infections, dectin-1 con) or patients homozygous for the mutation (dectin-1 hom). Using this methodology in medium enriched with 10% human serum, phagocytosis receptors such as Fc-receptors or complement-mediated binding are apparently sufficient to compensate for the absence of dectin-1. Results are presented as means  $\pm$  SD of four experiments.

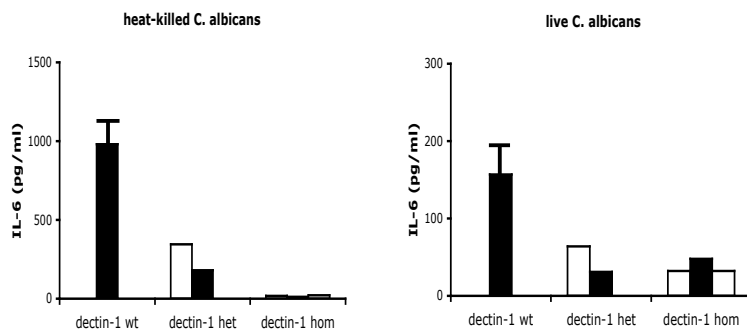
**Supplementary Figure S3. Dectin-1 mutation in a family with mucocutaneous fungal infections.** A. The nucleotide change (A > C) in exon 6 is shown in a person with the functional dectin-1 receptor (wild type) and two individuals having the stop mutation in heterozygote or homozygote state. B. Modelling of the mutated dectin-1 receptor. The missing nine aminoacids are depicted in red, the diagram showing the loss of a disulfide bridge and a Ca<sup>2+</sup>-binding site.

**Supplementary Figure S4. Dectin-1 mutation leads to functional defects.** A. IL-17 production capacity of PBMC from healthy volunteers (dectin-1 wt), patients heterozygous (dectin-1 het), or patients homozygous for the mutation (dectin-1 hom) stimulated for 5 days with heat-killed *C. albicans*. B. Only wild-type, but not mutated, isoforms A or B of dectin-1 were able to mediate binding of zymosan to the transfected NIH3T3 cells. The specificity of the binding was demonstrated by its inhibition with the dectin-1 antagonist laminarin. Representative results of five independent experiments are presented. C. The variant dectin-1 isoforms A (dectin-1A<sub>mut</sub>) and isoform B (dectin1-B<sub>mut</sub>) were poorly expressed on the surface of transfected NIHT3T cells, when compared to wild-type dectin-1 isoforms. D. The pattern of transcription of the dectin-1 isoforms is similar between healthy individuals with wild-type dectin-1 gene and patients homozygous for the early stop codon mutation. E. Extracellular FACS staining of monocytes (Mo) and neutrophils (PMN) isolated from healthy volunteers (dectin-1 con) or patients homozygous for the mutation (dectin-1 hom).

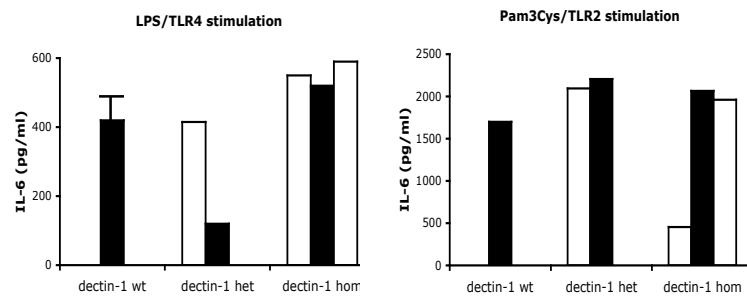
**Supplementary Figure S5. Dectin-1 stop mutation/polymorphism and haplotypes in different populations.** A. Phylogenetic representation of the dectin-1 receptor aminoacid area surrounding the Tyr238Stop, shown in green, in the mammalian lineage showing that the location of the mutation is conserved. B. Dectin-1 allele haplotype network of 1422 Caucasian (2844 haplotypes) and 171 African (342 haplotypes) individuals. Each circle represents a unique haplotype node and colours represent the different populations. Green is used for Caucasian haplotypes, Blue for African, and black for the ancient haplotype according to the dpSNP information. Circles with red outline contain the two haplotypes containing the stop polymorphism. The red circle following the ancient haplotype represents a vector node and does not represent an observed haplotype. Area of all circles indicates the amount of alleles with that haplotype. At the lower part, which includes the two haplotypes containing the stop polymorphism, SNP rs numbers that change between the nodes are presented. C. The two haplotypes containing the 238Stop mutations. Only the first three SNPs differ between these haplotypes, which are located after exon 6.

## Supplementary Figure S1

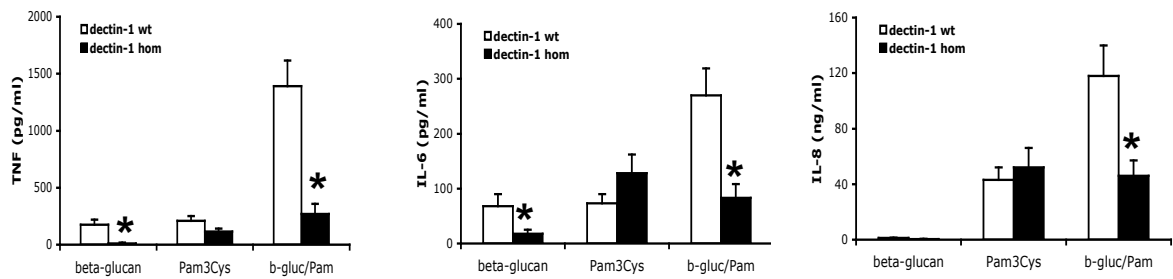
### A



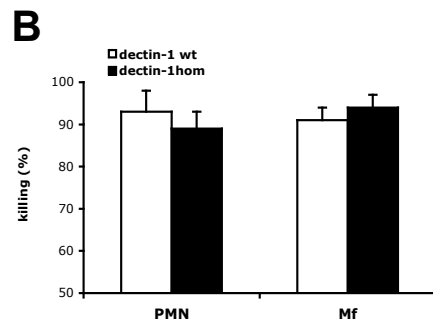
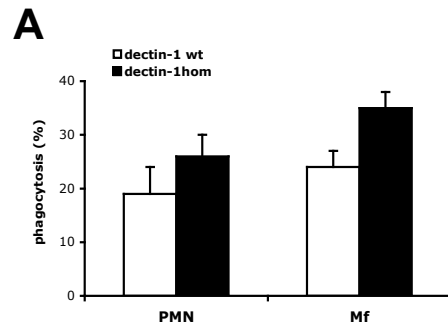
### B



### C

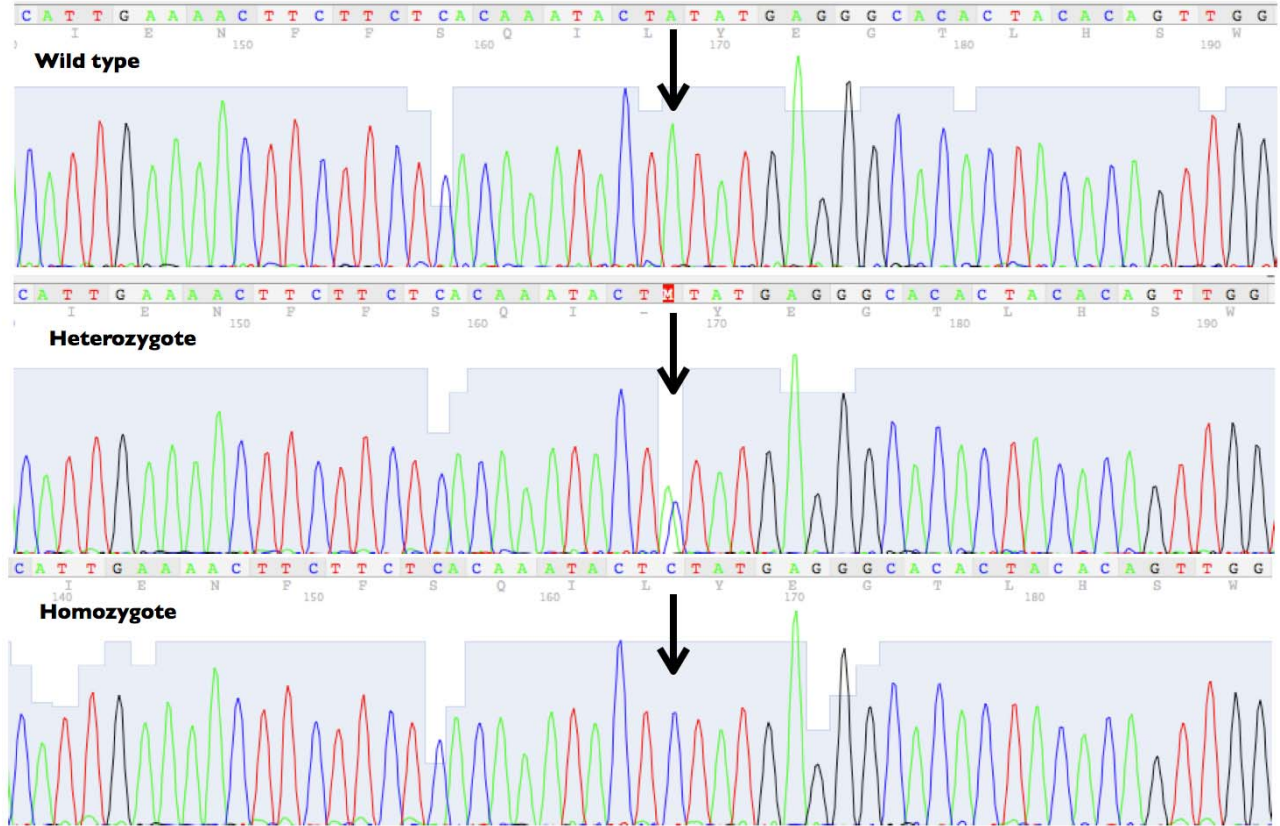


## Supplementary Figure S2

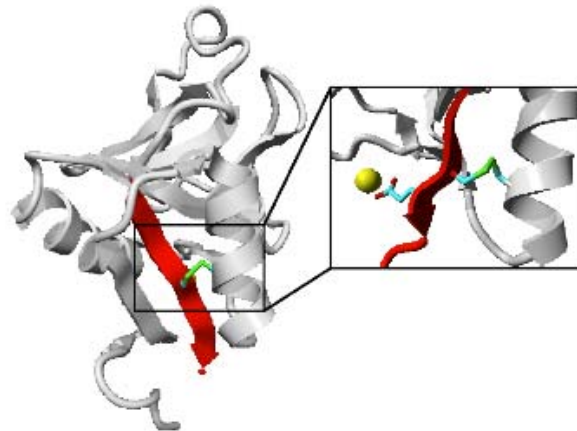


# Supplementary Figure S3

## A

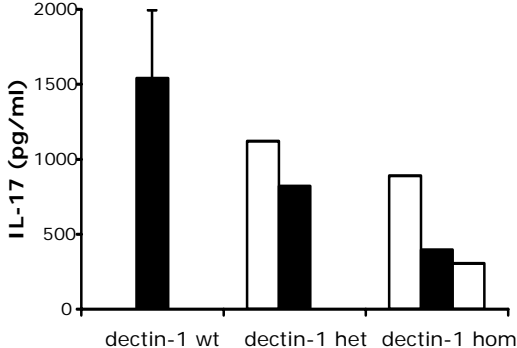


## B

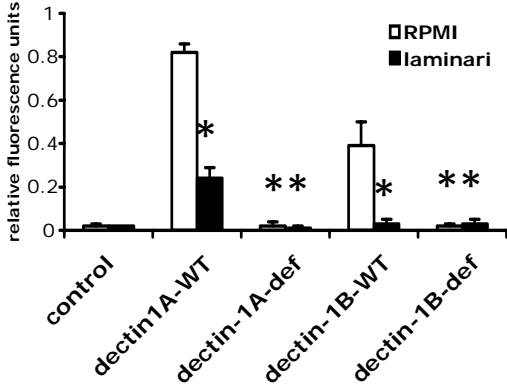


# Supplementary Figure S4

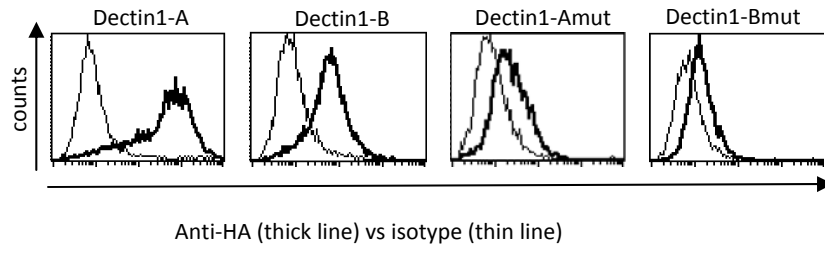
A



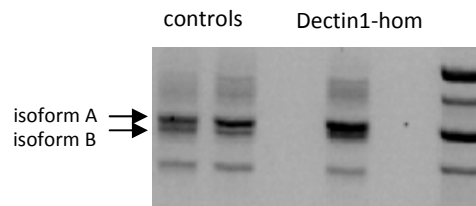
B



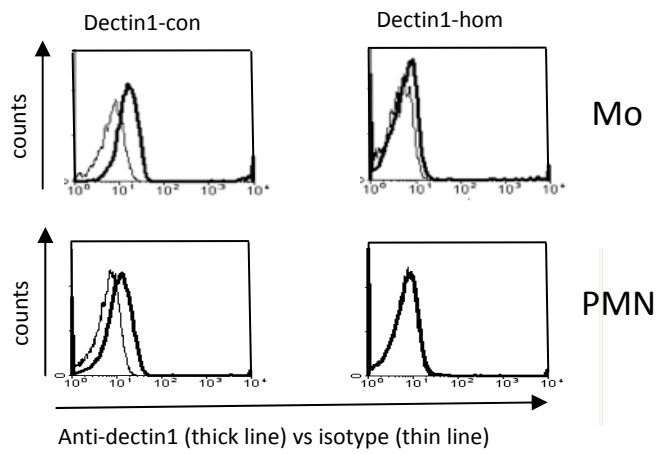
**C**



**D**

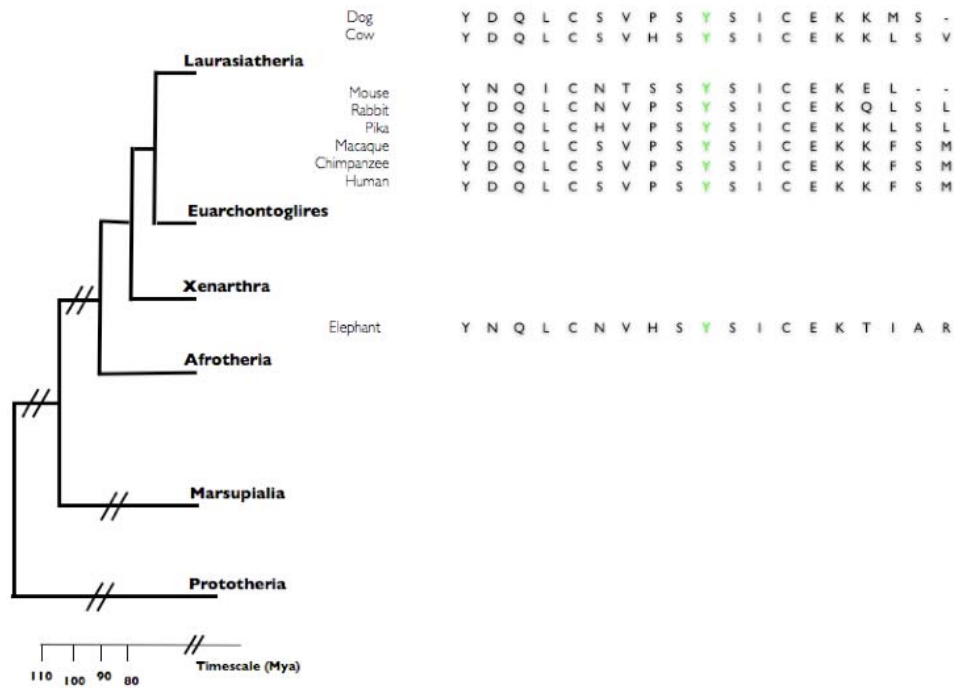


**E**

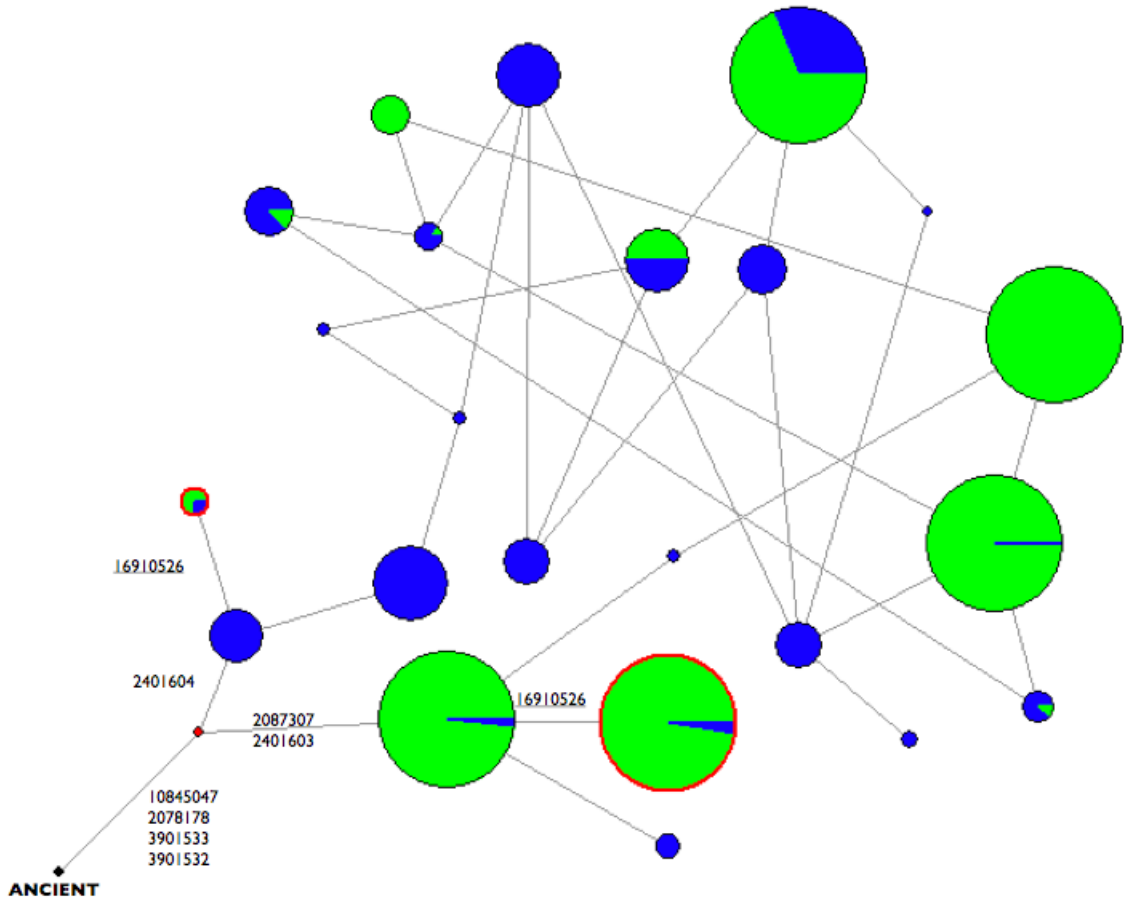


# Supplementary Figure S5

A



**B**



C

